The role of the central ghrelin system in reward from food and chemical drugs

Suzanne L Dickson, Emil Egecioglu, Sara Landgren, Karolina P Skibicka, Jörgen A. Engel, Elisabet Jerlhag

Title: The role of the central ghrelin system in reward from food and chemical drugs

Authors: Suzanne L Dickson, Emil Egecioglu, Sara Landgren, Karolina P Skibicka, Jörgen A. Engel, Elisabet Jerlhag

PII: S0303-7207(11)00154-7
DOI: doi:10.1016/j.mce.2011.02.017
Reference: MCE 7780

To appear in: *Molecular and Cellular Endocrinology*

Received date: 7-2-2011
Revised date: 17-2-2011
Accepted date: 17-2-2011

Please cite this article as: Dickson, S.L., Egecioglu, E., Landgren, S., Skibicka, K.P., Engel, J.A., Jerlhag, E., The role of the central ghrelin system in reward from food and chemical drugs, *Molecular and Cellular Endocrinology* (2010), doi:10.1016/j.mce.2011.02.017

The role of the central ghrelin system in reward from food and chemical drugs

Suzanne L Dickson[1], Emil Egecioglu[1], Sara Landgren[2], Karolina P Skibicka[1], Jörgen A. Engel[2], Elisabet Jerlhag[2]

Departments of [1]Physiology and [2]Pharmacology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sweden.

Correspondence to:

Prof Suzanne L Dickson (Dept Physiology/Endocrinology) or Dr Elisabet Jerlhag (Dept Pharmacology), The Sahlgrenska Academy at the University of Gothenburg, Medicinaregatan 11, SE-436 44 Gothenburg, Sweden. Email: suzanne.dickson@gu.se or elisabet.jerlhag@pharm.gu.se

Abstract

Here we review recent advances that identify a role for the central ghrelin signalling system in reward from both natural rewards (such as food) and artificial rewards (that include alcohol and drugs of abuse). Whereas ghrelin emerged as a stomach-derived hormone involved in energy balance, hunger and meal initiation via hypothalamic circuits, it now seems clear that it also has a role in motivated reward-driven behaviours via activation of the so-called "cholinergic-dopaminergic reward link". This reward link comprises a dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens together with a cholinergic input, arising primarily from the laterodorsal tegmental area. Ghrelin administration into the VTA or LDTg activates the "cholinergic-dopaminergic" reward link, suggesting that ghrelin may increase the incentive value of motivated behaviours such as reward-seeking behaviour ("wanting" or "incentive motivation"). Further, direct injection of ghrelin into the brain ventricles or into the VTA increases the consumption of rewarding foods as well as alcohol in mice and rats. Studies in rodents show beneficial effects of ghrelin receptor (GHS-R1A) antagonists to suppress the intake of palatable food, to reduce preference for caloric foods, to suppress food reward and motivated behaviour for food. They have also been shown to reduce alcohol consumption, suppress reward induced by alcohol, cocaine and amphetamine. Further, variations in the GHS-R1A and pro-ghrelin genes have been associated with high alcohol consumption, smoking and increased weight in alcohol dependent individuals as well as with bulimia nervosa and obesity. Thus, the central ghrelin signalling system interfaces neurobiological circuits involved in reward from food

as well as chemical drugs; agents that directly or indirectly suppress this system emerge as potential candidate drugs for suppressing problematic over-eating that leads to obesity as well as for the treatment of substance use disorder.

The central ghrelin signalling system

The "central ghrelin signalling system" is recognised as an important CNS target for the control of food intake (Wren et al. 2000) and energy balance (Lall et al. 2001; Tschöp et al. 2000). Here we review the emerging concept that this system operates at the interface between neurobiological circuits involved in appetite and reward, increasing the incentive motivational value of both natural rewards (such as food) and artificial rewards (such as drugs of abuse).

We have coined the term "central ghrelin signalling system" to describe those CNS pathways that are directly or indirectly affected as a result of changes in stimulation of the known ghrelin receptor, GHS-R1A (growth hormone secretagogue receptor 1A). GHS-R1A is present in a number of brain areas that include the hypothalamus, brainstem, tegmentum and hippocampus (Guan et al. 1997; Howard et al. 1996; Zigman et al. 2006). By using the term "central ghrelin signalling system" we acknowledge the interesting pharmacology of this receptor, namely, that it has constitutive activity in the absence of ghrelin ligand (Holst et al. 2003; Holst and Schwartz 2004). It follows that the activity of the receptor is not only dependent on the gut-brain signal provided by

ghrelin. Indeed, the extent to which circulating ghrelin provides a physiological relevant signal for activation of this receptor, poses an interesting question. Moreover, the activity of GHS-R1A can be suppressed not only through pharmacological antagonism of ghrelin's effects but also independently of ghrelin (eg by using inverse agonists) (Mokrosinski and Holst 2010). The "central ghrelin signalling system" emerges therefore as a key physiological system implicitly involved in the control of food intake and reward behaviour and also as a potential therapeutic target for the control of obesity as well as disorders of the reward system that include eating disorders and substance use disorders.

Interest in GHS-R1A as a therapeutic target began in the 1980s when a peptide called growth hormone-releasing peptide 6 (GHRP-6), the canonical member of a class of synthetic molecules known as growth hormone secretagogues (GHS), was identified as a potent stimulator of the hypothalamo-pituitary growth axis (Bowers et al. 1984). The receptor for these ligands, GHS-R1A, was first described some years later by the group at Merck & Co (Howard et al. 1996). Like many pharmaceutical companies at that time, they were developing potent agonists for improving growth hormone status. Given that growth hormone is lipolytic, there was little reason to link GHS-R1A to food intake or adiposity. The first hint that the central ghrelin signalling system is a relevant target for appetite and energy balance came from an unexpected observation in our group that the hypothalamic cells activated by GHRP-6 include not only the growth hormone-releasing hormone neurons in the arcuate nucleus but also the orexigenic NPY neurons in

this region (Dickson et al. 1993; Dickson and Luckman 1997). These NPY cells (that also co-express another orexigenic peptide, agouti-related peptide) are now known to form a key component of the hypothalamic energy homeostatic circuits, receiving and integrating information also from many circulating satiety-regulating signals such as leptin and insulin (Cone et al. 2001; Hewson et al. 2002).

The physiological role of ghrelin: from hunger to reward-seeking

Soon after the discovery of ghrelin, the first endogenous ligand for GHS-R1A (Kojima et al. 1999), it became clear that this receptor is also a potentially interesting target for controlling food intake and obesity. In rodents, acute injection of ghrelin, peripherally or centrally, induces a rapid orexigenic response (Asakawa et al. 2001; Wren et al. 2000). It was also found that chronic stimulation of this receptor by ghrelin (Tschöp et al. 2000) or by synthetic growth hormone secretagogues (Lall et al. 2001) increases fat mass in rodents, by a mechanism that is independent of the hypothalamo-pituitary growth axis and, unexpectedly, did not appear to involve a hyperphagic response. Indeed, it has been proposed that ghrelin decreases fat utilization (Theander-Carrillo et al. 2006; Tschöp et al. 2000), implying that the CNS circuits mediating ghrelin's acute orexigenic effects differ, at least in part, from those involved in fat accumulation. We noticed previously that ghrelin's orexigenic effects decline after a few days of repeated peripheral ghrelin injection (Dornonville de la Cour et al. 2005). However, in another chronic study, in which rats received a 2 week infusion of ghrelin into the brain ventricles, we were surprised to detect a clear orexigenic response (Salomé et al. 2009), suggesting that under certain

experimental/physiological conditions, the orexigenic ghrelin-responsive networks remain ghrelin-sensitive during chronic exposure.

Ghrelin levels increase pre-prandially (Cummings et al. 2001) and have been shown to correlate with hunger scores in healthy subjects (Cummings et al. 2004) indicating that, at least in normal physiology, acute changes of ghrelin may have a role in the decision to eat and/or serve as a circulating hunger hormone. Studies in rodents exploring the precise parenchymal targets for ghrelin's orexigenic effects have identified a number of candidate sites: orexigenic responses have been observed when injected into discrete brain areas that include the hypothalamus (arcuate nucleus, ventromedial nucleus, dorsomedial nucleus, paraventricular nucleus, lateral hypothalamus)(Olszewski et al. 2003a; Olszewski et al. 2003b; Wren et al. 2001), the nucleus tractus solitarius of the brainstem (Faulconbridge et al. 2003), the central nucleus of the amygdala (Olszewski et al. 2003) and mesolimbic reward areas (the ventral tegmental area (VTA) and nucleus accumbens (N.Acc) (Egecioglu et al. 2010; Naleid et al. 2005). Activation of hypothalamic and brainstem areas could be of importance for ghrelin's effects on homeostatic feeding that is driven by metabolic need. However, ghrelin may also enhance non-homeostatic feeding by interacting with key reward systems (Egecioglu et al. 2010; Perello et al. 2010; Skibicka et al. 2011a; Skibicka et al. 2011b). The reward system, specifically the mesolimbic dopamine system, mediates a state of well-being of natural and chemical reinforcers; it enhances motivation behaviours such as food-seeking and is involved in the development of addiction to drugs of abuse (Engel et al. 1988; Hansen et al. 1991;

Schultz et al. 1997). When consumed in excess and over time food can cause the same brain neuroadaptations as drug abuse (Grigson 2002). Indeed, human imaging studies show that there is an underlying disruption in the reward systems in the brain (Holden 2001; Potenza et al. 2003; Volkow et al. 2003; Volkow et al. 2003), as well as in brain regions important for inhibitory control (Volkow et al. 2003) after prolonged use of both natural and chemical reinforcers. Moreover, there are behavioural parallels between compulsive overeating and drug dependence such as loss of control (Davis and Woodside 2002). Therefore "behavioural" addictions, such as compulsive overeating, gambling and compulsive shopping, have been included in the definition of addiction and are, together with drug dependence, called "addictive behaviours'".

The identification of the reward circuits as a key target for ghrelin has therefore led to the unexpected discovery that the central ghrelin signalling system is required for reward induced by addictive drugs (Jerlhag et al. 2009; Kaur and Ryabinin 2010; Tessari et al. 2007; Wellman et al. 2005) as well as palatable food (Egecioglu et al. 2010; Perello et al. 2010; Skibicka et al. 2011a; Skibicka et al. 2011b). The ghrelin receptor, GHS-R1A, has emerged therefore as a relevant therapeutic target for addictive behaviours.

The central ghrelin signalling system integrates with a key reward pathway

The mesolimbic dopamine pathway (Figure 1) from the VTA to the N.Acc, that has a rather well-established role in incentive motivation (ie wanting)(Berridge and Robinson 2003), is clearly an important component of the central ghrelin-responsive network. Ghrelin

receptors are present in the VTA (Guan et al. 1997; Zigman et al. 2006), including a sub-population of dopamine cells in this region (Abizaid et al. 2006). Both central and intra-VTA administration of ghrelin induces an increase in accumbal dopamine release and also induces a locomotor stimulation (Jerlhag et al. 2006a; Jerlhag et al. 2007) as well as an increase in dopamine turnover in N.Acc (Abizaid et al. 2006). Peripherally injected ghrelin also stimulates the mesolimbic dopamine system (Jerlhag 2008; Quarta et al. 2009) suggesting that peripherally produced ghrelin is able to access (or indirectly activate) the mesolimbic reward circuits. Interestingly, administration of a GHS-R1A antagonist into the VTA blocks the ability of ghrelin to increase food intake (Abizaid et al. 2006) and to release dopamine in the N.Acc (Jerlhag et al. 2011) suggesting that ghrelin activates GHS-R1A expressed on dopaminergic cell bodies in the VTA. Given that accumbal dopamine release appears to mediate the rewarding properties of incentives (eg food and alcohol) (Engel et al. 1988; Robinson and Berridge 1993; Wise and Bozarth 1987), the collective data showing that ghrelin targets this dopamine system implicates GHS-R1A directly in the reward mechanism.

The mesolimbic dopamine system forms together with the cholinergic projection from the laterodorsal tegmental area (LDTg) to the VTA the cholinergic-dopaminergic reward link (Figure 1). This reward link is activated by natural as well as chemical reinforcers, as reviewed previously (Larsson and Engel 2004). Involvement of this reward link in drug-induced as well as natural reward has been implied, thus food as well as alcohol intake increases the ventral tegmental acetylcholine levels as well as accumbal dopamine

levels (Hernandez and Hoebel 1988; Larsson et al. 2005; Rada et al. 2000). GHS-R1A is expressed in the LDTg (Guan et al. 1997), specifically on cholinergic cells (Dickson et al. 2010), suggesting that ghrelin may have direct effects in this reward node. We have found that local administration of ghrelin into the LDTg activates parameters associated with reward, namely locomotor stimulation and accumbal dopamine release (Jerlhag et al. 2007). Moreover, these effects of ghrelin were blocked by peripheral or intra-VTA administration of an unselective nicotinic cholinergic antagonist, mecamylamine (Jerlhag et al. 2006a; Jerlhag et al. 2008). Additionally, peripheral injection of mecamylamine has been shown to block the ability of ghrelin, locally administered into the VTA, to increase food intake (Dickson et al. 2010). Collectively, these studies demonstrate that ghrelin activates the cholinergic-dopaminergic reward link and thereby may increase the incentive salience of motivated behaviours such as reward seeking. Further, by using selective pharmacological antagonists of nicotinic acetylcholine receptor subtypes it was shown that the ability of ghrelin to activate the cholinergic-dopaminergic reward link is mediated via specific subtypes of the nicotinic acetylcholine receptor, namely the α3β2*, α6* and β3* (Jerlhag et al. 2008). Interestingly, these subtypes also mediate the rewarding properties of alcohol, alcohol cues as well as alcohol intake in rodents (Ericson et al. 2009; Jerlhag et al. 2006b; Larsson and Engel 2004; Löf et al. 2007; Steensland et al. 2007). These data are verified in clinical tests; thus blocking these subtypes, by using varenicline, reduces the intake of alcohol in heavy drinking smokers (McKee et al. 2009) and one haplotype of the α6* gene is associated with heavy alcohol use (Landgren et al. 2009). Furthermore, it has

been shown that excessive alcohol intake in rats consuming alcohol for a long period of time causes a release of acetylcholine in the VTA followed by an increase in accumbal dopamine levels, showing that alcohol like ghrelin activates the cholinergic-dopaminergic reward link (Larsson et al. 2005). Taken together, there appears to be neurochemical analogies and overlaps between ghrelin and alcohol, where the cholinergic-dopaminergic reward link is the common denominator.

The activity of dopaminergic neurons in the VTA are mediated via various afferents and within the VTA GHS-R1A is present, not only on the dopaminergic cells, but also on pre-synaptic afferents (Abizaid et al. 2006) that could mediate the ability of ghrelin to activate the reward systems. Specifically, it has been found that the ability of ghrelin to increase the locomotor activity, accumbal dopamine release and condition a place preference (ie associate reward with a ghrelin-paired environment) was attenuated by a non-selective glutamate NMDA receptor antagonist (AP5) (Jerlhag et al. 2011). Supportively, the ability of ghrelin to increase the electrical activity of dopaminergic neurons in the VTA appears to be dependent on the excitatory glutamatergic input, and also blockade of NMDA receptors in the VTA reduces food-induced accumbal dopamine release (Abizaid et al. 2006; Taber and Fibiger 1997). Furthermore, NMDA receptors have also been shown to mediate the accumbal dopamine release observed when animals consume food after ghrelin administration (Kawahara et al. 2009). By contrast, we were unable to demonstrate effects of either an opioid receptor antagonist or an orexin receptor A antagonist on the ability of ghrelin to activate the mesolimbic

dopamine system (Jerlhag et al. 2011). Albeit, central orexin signalling is important for ghrelin-induced food intake and for appetitive behaviour for high fat feeding (Perello et al. 2010; Toshinai et al. 2003), implying that the ghrelin-induced reward versus ghrelin-induced food intake are regulated, at least in part, via different mechanisms. Given that the plasma levels of ghrelin are elevated in association with addictive behaviours (see below), future therapeutic targets for these disorders may include agents such as nicotinic acetylcholine receptor or glutamate receptor antagonists acting at the level of the cholinergic-dopaminergic reward link.

The central ghrelin signalling system is required for reward from drugs of abuse, including alcohol.

Alcohol dependence, one of the major causes of death, is a chronic relapsing disease and is also a major cost for the society. Available treatments include medical, psychosocial as well as social interventions, which may help to increase time to relapse and to reduce the intake of alcohol. Alcohol dependence is a heterogeneous disorder where several signalling systems play important roles. By understanding the complex mechanisms underlying this disease new treatment strategies may be developed. While ghrelin was gaining status as a circulating hunger hormone, the work pinpointing the midbrain dopamine system as a target for ghrelin, led us to hypothesise that this system may have a role that extends beyond regulation of food intake and of energy balance homeostasis to include reward seeking behaviour, not only for rewarding foods but also for other reward reinforcers such as alcohol and other drugs of abuse. Interestingly, we

have now shown that ghrelin and its receptor have a role in drug-induced reward and specifically in alcohol dependence.

With regard to GHS-R1A, we showed that suppression of the GHS-R1A, using pharmacological or genetical approaches, reduces the rewarding properties of alcohol as measured by locomotor activity, accumbal dopamine release and conditioned place preference (ie associate reward with an alcohol-paired environment) in mice. Moreover, central or peripheral administration of the GHS-R1A antagonists (BIM28163 or JMV2959 respectively) reduces the intake of alcohol in mice consuming moderate amounts of alcohol (Jerlhag et al. 2009). These findings were later independently verified in studies showing that a GHS-R1A antagonist (D-Lys3-GHRP-6) reduces the intake of alcohol in mice (Kaur and Ryabinin 2010). The GHS-R1A antagonist (JMV2959) has also been found to reduce the intake of alcohol using the intermittent alcohol consumption paradigm as well as operant self-administration model in high alcohol-preferring rats (Landgren et al. 2011). Given that the GHS-R1A regulates the intake and reward from alcohol, the question arose regarding the extent to which the central ghrelin signalling system could be important for reinforcement from other drugs of abuse. Thus, peripheral administration of a GHS-R1A antagonist attenuates the rewarding properties of amphetamine and cocaine as measured by locomotor stimulation, accumbal dopamine release and conditioned place preference (Jerlhag et al. 2010a). Clinical data also suggest a role for the GHS-R1A in regulating the intake of addictive drugs. Thus associations between one single SNP and haplotypes in the GHS-R1A gene and alcohol

consumption, increased weight as well as smoking in alcohol dependant individuals have been found (Landgren et al. 2008; Landgren et al. 2010).

The endogenous peptide ghrelin also appears to have a role in drug-induced reward. Specifically, ghrelin administration (icv or into the LDTg or VTA) increases the intake of alcohol in mice and the ability of alcohol to induce a locomotor stimulation, accumbal dopamine release and to condition a place preference is reduced in ghrelin knockout mice (Jerlhag et al. 2009). These effects are most likely mediated at the level of the cholinergic-dopaminergic reward link since hypothalamic administration of ghrelin does not influence the intake of alcohol in rats (Schneider et al. 2007). Moreover, the effect of ghrelin is more pronounced in rodents exposed to alcohol since peripheral ghrelin administration to alcohol naïve rats only slightly increases the alcohol intake (Lyons et al. 2008). Additionally, ghrelin augments cocaine-induced reward as measured by locomotor activity and conditioned place preference, and elevated ghrelin levels are associated with cocaine-seeking in rats (Davis et al. 2007; Tessari et al. 2007; Wellman et al. 2005). Food restriction, that increases ghrelin levels (Gualillo et al. 2002), augments cocaine- as well as amphetamine-induced locomotor stimulation, enhances cocaine-seeking behaviour and increases the self-administration of cocaine or amphetamine in rats (Carroll et al. 1979). In human genetic studies a ghrelin gene haplotype has been associated with paternal heredity of alcohol-use disorder (Landgren et al. 2010) and with increased weight in alcohol dependent individuals (Landgren et al. 2008). After drinking alcohol the plasma levels of ghrelin decrease in healthy controls (Calissendorff

et al. 2005; Calissendorff et al. 2006; Zimmermann et al. 2007) which is a similar effect to the reduced plasma levels of ghrelin post-prandially (Tschöp et al. 2001). The relationship between plasma ghrelin levels and alcohol dependence in humans is somewhat unclear, where some studies show increased whereas some show decreased levels of ghrelin (Addolorato et al. 2006; Badaoui et al. 2008; Hillemacher et al. 2007; Kim et al. 2005; Kraus et al. 2005). These apparent deviant results may depend on the heterogeneity of the disease and also on the time frame when the samples have been taken. Thus, it has been shown that ghrelin increases during the initial phase of abstinence but does not differ from healthy controls later during abstinence (Wurst et al. 2007), and that elevated ghrelin levels are associated with craving during alcohol abstinence (Addolorato et al. 2006) (for review see Leggio 2010).

Collectively these clinical and preclinical findings suggest that ghrelin as well as the GHS-R1A may regulate both the intake of and search for rewarding substances, and implies that treating patients with alcohol dependence with pharmacological agents interfering with the central ghrelin signalling system may have beneficial effects.

The central ghrelin signalling system is required for reward from food

In normal physiology, it seems likely that ghrelin does indeed operate as a circulating hunger hormone, conferring information to the brain about energy deficit and then interacting with key brain circuits to manifest a coordinated feeding response. This is achieved, in part, through activation of the aforementioned hypothalamic and

brainstem circuits involved in energy homeostasis. Specifically, it has been shown that ghrelin increases food intake via activation of homeostatic feeding circuits that include orexigenic circuits in the arcuate nucleus (Figure 1) (Cowley et al. 2003). It also seems clear that ghrelin activates pathways involved in non-homeostatic feeding, thereby influencing appetite behaviour and motivational aspects of food intake (including all behaviours that precede food ingestion). For a review on this topic, we refer the reader to Skibicka and Dickson 2011. In normal weight healthy volunteers, ghrelin has been shown to increase hunger scores, to enhance food palatability and to increase caloric intake in a free-feeding buffet situation (Cummings et al. 2004; Druce et al. 2006; Wren et al. 2001). One recent study of food economics reported that ghrelin even increases the amount of money an individual is prepared to pay for individual food items (Tang et al. 2011).

In rodents, ghrelin's orexigenic effects extend to food foraging and food hoarding (Keen-Rhinehart and Bartness 2005a; Keen-Rhinehart and Bartness 2005b), food anticipation (Blum et al. 2009; Verhagen et al. 2010), food preference, food reward and food motivation (Abizaid et al. 2006; Egecioglu et al. 2010; Perello et al. 2010; Skibicka et al. 2011a). Peripheral ghrelin appears to enhance sweet taste food consumption and preference, regardless of its caloric content (Disse et al. 2010). Direct administration of ghrelin into the brain ventricles or into the VTA stimulates food intake, of both normal chow and palatable food (Abizaid et al. 2006; Egecioglu et al. 2010; Naleid et al. 2005; Skibicka et al. 2011a). Indeed, the fact that ghrelin can achieve this even in satiated

animals suggested that the VTA is an important site for ghrelin's orexigenic effects. Consistent with this, we found that central intraventricular (Skibicka et al. 2011a) or intra-VTA (Skibicka et al. 2011b) injection of ghrelin to satiated rats increased motivation for a sucrose reward, assessed in an operant conditioning paradigm. In these studies animals show motivated behaviour by working increasingly hard (pressing a lever) to obtain a sweet treat. Thus, exogenous ghrelin not only initiates food intake but also, through enhancing motivated behaviour for rewarding food, enhances feeding behaviour and the amount of food consumed.

The orexigenic and adipogenic effects of ghrelin can be suppressed by ghrelin antagonists (Asakawa et al. 2003; Esler et al. 2007; Salomé et al. 2009a; Salomé et al. 2009b). One week peripheral treatment of rats with the GHS-R1A antagonist, JMV2959, suppressed preference for palatable/rewarding food, and they did not gain as much weight as the vehicle-treated control rats (Egecioglu et al. 2010). In this study, the ability of rewarding sweet treats to condition a place preference was also suppressed by the antagonists. In this powerful test of reward, the animals no longer show preference for the food (sweet treat)-paired environment, suggesting that the GHS-R1A antagonists suppress food reward. Moreover, we found that motivated behaviour for food reward can also be suppressed by intra-ventricular or intra-VTA administration of JMV2959 (Skibicka et al. 2011a; Skibicka et al. 2011b). Thus, as reviewed elsewhere (Egecioglu et al. 2011; Skibicka and Dickson 2011), GHS-R1A provides a potential target for the treatment of problematic over-eating. Indeed, it will be important to establish how the

ghrelin-responsive circuits adapt during the development of diet-induced obesity (Lindqvist et al. 2005; Briggs et al. 2010), and whether GHS-R1A antagonists could provide an effective therapy for this disease area.

The emerging neurobiology of central ghrelin signalling indicates that it may serve as a common denominator to enhance motivated behaviour for natural and artificial reward. This hypothesis is substantiated further by human functional magnetic resonance imaging in which ghrelin administration to healthy volunteers altered the brain response to visual food cues in areas such as the ventral striatum (Malik 2008). Several studies have demonstrated an association between anomalous plasma ghrelin levels and aberrant eating patterns. It has been suggested that a hyperghrelinemia may have a role in the pathophysiology of binge eating (i.e. compulsive overeating), at least in bulimic, anorectic and Prader-Willi patients (a genetic disorder affecting cognitive function that is associated with obesity). Children with Prader-Willi syndrome, who have not yet developed the core symptoms, have normal plasma levels of ghrelin (Erdie-Lalena et al. 2006), whereas the ghrelin levels increase along with development of hyperphagia and obesity (Cummings et al. 2002; Erdie-Lalena et al. 2006). Similarly, anorectic and bulimic patients of the binge type have higher levels of ghrelin in the plasma than their non-binging counterparts, and the frequencies of binging correlate positively with plasma ghrelin levels (Tanaka et al. 2003).

Various polymorphisms in the GHS-R1 gene have been associated with bulimia nervosa

and obesity (Baessler et al. 2005; Holst and Schwartz 2006; Wang et al. 2004;). Moreover, different pro-ghrelin gene polymorphisms have been linked to obesity, and to bulimia nervosa purging individuals (Ando et al. 2006; Gueorguiev et al. 2002; Korbonits et al. 2002; Ukkola et al. 2001; Vivenza et al. 2004; Vartiainen et al. 2006) as well as methamphetamine withdrawal (Yoon et al. 2005).

Thus, GHS-R1A emerges as a potential therapeutic target to suppress food reward and motivated behaviour for food, with possibilities for treating eating disorders that lead to obesity, especially those for which a dysfunctional reward system have been implicated (eg binging behaviour, compulsive over-eating).

Conclusions

The exact circuitry and mechanisms through which ghrelin modulates the intake and seeking of rewards remains to be further elucidated, but likely involves actions at the level of the cholinergic-dopaminergic reward system. GHS-R1A is expressed pre- and post-synaptically in the VTA (Abizaid et al. 2006) as well as on cholinergic neurons in the LDTg (Dickson et al. 2010). Ghrelin injection into these discrete brain regions increases accumbal dopamine (Jerlhag et al. 2007) increases alcohol intake (Jerlhag et al. 2009) and food intake as well as motivated behaviour for palatable foods (Egecioglu et al. 2010; Skibicka et al. 2011b). Indeed, pharmacological manipulations within the VTA reduce reward induced by ghrelin, additive drugs as well as for palatable food (Egecioglu et al. 2010; Jerlhag et al. 2009; Jerlhag et al. 2010a; Skibicka et al. 2011b).

Extensive literature, as discussed herein, shows that suppression of central ghrelin signalling attenuates natural as well as chemical reinforcement as well as seeking for rewards. We suggest that the central ghrelin signalling system alters the set point of the dopaminergic neurons in the VTA and thereby enhances the ability of reward reinforcers such as food, alcohol or addictive drugs, to activate the midbrain dopamine reward system. It remains to be determined whether it is ghrelin itself that provides the signal to enhance the reward mechanism as the ghrelin receptor, GHS-R1A, has constitutive activity in absence of ligand (Holst et al. 2003). Perhaps the GHS-R1A is regulated independently of ghrelin, for example, via heterodimerization to the dopamine D1-like receptor (Jiang et al. 2006). Interestingly, a role for D1 receptors in mediating alcohol intake, preference and oral self-administration in rodents has been found (Dyr et al. 1993; El-Ghundi et al. 1998). It is not yet known how the dopamine D1 receptor influences central ghrelin signalling and the physiological relevance of this dimerization remains to be determined.

Interestingly, there appears to be co-morbidities between eating disorders (specifically binge eating) and drug dependence (eg Anzengruber et al. 2006; Bulik 1991) and human imaging studies reveal an underlying disruption in the reward systems in addictive behaviours, including alcohol use disorder and binge eating (Volkow et al. 2003; Volkow and Li 2004). Therefore we suggest that similar mechanisms may play a role in multiple addictions and that central ghrelin signalling at the level of the cholinergic-dopaminergic

reward link play an important role. Indeed, SNPs and haplotypes of both the pro-ghrelin and GHS-R1A genes have been associated with increased weight in alcohol dependent individuals (Landgren et al. 2008). Central ghrelin signalling has over the last years been shown to mediate the reward from alcohol (Jerlhag et al. 2009; Jerlhag et al. 2010b), cocaine, amphetamine (Jerlhag et al. 2010a; Wellman et al. 2005; Tessari et al. 2007), and palatable/rewarding food (Egecioglu et al. 2010; Perello et al. 2010; Skibicka et al. 2011a; Skibicka et al. 2011b). Collectively these studies imply that central ghrelin signalling, including the GHS-R1A may constitute a novel target for development of treatment strategies for addictive behaviours.

Acknowledgment

Supported by the EU (FP7- HEALTH- 2009- 241592, FP7-KBBE-2009-3-245009; FP7-KBBE-2010-266408), Swedish Medical Research Council (K2007-54X-20328–013, K2006-21X-04247-33-3, 2009-S266, and 2009-2782), ALF/LUA grants from the Sahlgrenska Hospital Göteborg (SU7601, 7136, 7341, ALFGBG-138741, -148251), the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly, The Swedish brain foundation, the foundations of Wilhelm and Martina Lundgren, Knut and Alice Wallenberg, The Adlerbert Research, Thuring's, Längmanska art, Torsten and Ragnar Söderberg, Magnus Bergvall foundation, The Swedish Society of Medicine and the Swedish Foundation for Strategic Research to Sahlgrenska Center for Cardiovascular and Metabolic Research (A305-188) .

References

Abizaid, A., Liu, Z.W., Andrews, Z.B., Shanabrough, M., Borok, E., Elsworth, J.D., Roth, R.H., Sleeman, M.W., Picciotto, M.R., Tschop, M.H., Gao, X.B. and Horvath, T.L. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite Journal of Clinical Investigation **116**(12): 3229-3239.

Addolorato, G., Capristo, E., Leggio, L., Ferrulli, A., Abenavoli, L., Malandrino, N., Domenicali, M. and Gasbarrini, G. (2006). Relationship between ghrelin levels, nutritional status and craving in current alcoholics Alcoholism-Clinical and Experimental Research **30**(9): 140a-140a.

Ando, T., Komaki, G., Naruo, T., Okabe, K., Takii, M., Kawai, K., Konjiki, F., Takei, M., Oka, T., Takeuchi, K., Masuda, A., Ozaki, N., Suematsu, H., Denda, K., Kurokawa, N., Itakura, K., Yamaguchi, C., Kono, M., Suzuki, T., Nakai, Y., Nishizono-Maher, A., Koide, M., Murakami, K., Nagamine, K., Tomita, Y., Ookuma, K., Tomita, K., Tonai, E., Ooshima, A., Ishikawa, T. and Ichimaru, Y. (2006). Possible role of preproghrelin gene polymorphisms in susceptibility to bulimia nervosa American Journal of Medical Genetics Part B-Neuropsychiatric Genetics **141B**(8): 929-934.

Anzengruber, D., Klump, K.L., Thornton, L., Brandt, H., Crawford, S., Fichter, M.M., Halmi, K.A., Johnson, C., Kaplan, A.S., LaVia, M., Mitchell, J., Strober, M., Woodside, D.B., Rotondo, A., Berrettini, W.H., Kaye, W.H. and Bulik, C.M. (2006). Smoking in eating disorders Eat Behav **7**(4): 291-299.

Asakawa, A., Inui, A., Kaga, T., Katsuura, G., Fujimiya, M., Fujino, M.A. and Kasuga, M. (2003). Antagonism of ghrelin receptor reduces food intake and body weight gain in mice Gut **52**(7): 947-952.

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., Makino, S., Fujimiya, M., Niijima, A., Fujino, M.A. and Kasuga, M. (2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin Gastroenterology **120**(2): 337-345.

Badaoui, A., De Saeger, C., Duchemin, J., Gihousse, D., de Timary, P. and Starkel, P. (2008). Alcohol dependence is associated with reduced plasma and fundic ghrelin levels European Journal of Clinical Investigation **38**(6): 397-403.

Baessler, A., Hasinoff, M.J., Fischer, M., Reinhard, W., Sonnenberg, G.E., Olivier, M., Erdmann, J., Schunkert, H., Doering, A., Jacob, H.J., Comuzzie, A.G., Kissebah, A.H. and Kwitek, A.E. (2005). Genetic linkage and association of the growth hormone secretagogue receptor (Ghrelin receptor) gene in human obesity Diabetes **54**(1): 259-267.

Berridge, K.C. and Robinson, T.E. (2003). Parsing reward Trends in Neurosciences **26**(9): 507-513.

Blum, I.D., Patterson, Z., Khazall, R., Lamont, E.W., Sleeman, M.W., Horvath, T.L. and Abizaid, A. (2009). Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice Neuroscience **164**(2): 351-359.

Bowers, C.Y., Momany, F.A., Reynolds, G.A. and Hong, A. (1984). On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone Endocrinology **114**(5): 1537-1545.

Briggs, D.I., Enriori, P.J., Lemus, M.B., Cowley, M.A. and Andrews, Z.B. (2010). Diet-induced obesity causes ghrelin resistance in arcuate NPY/AgRP neurons Endocrinology **151**(10): 4745-4755.

Bulik, C.M. (1991). Family histories of bulimic women with and without comorbid alcohol abuse or dependence Am J Psychiatry **148**(9): 1267-1268.

Calissendorff, J., Danielsson, O., Brismar, K. and Rojdmark, S. (2005). Inhibitory effect of alcohol on ghrelin secretion in normal man European Journal of Endocrinology **152**(5): 743-747.

Calissendorff, J., Danielsson, O., Brismar, K. and Rojdmark, S. (2006). Alcohol ingestion does not affect serum levels of peptide YY but decreases both total and octanoylated ghrelin levels in healthy subjects Metabolism-Clinical and Experimental **55**(12): 1625-1629.

Carroll, M.E., France, C.P. and Meisch, R.A. (1979). Food-Deprivation Increases Oral and Intravenous Drug Intake in Rats Science **205**(4403): 319-321.

Cone, R.D., Cowley, M.A., Butler, A.A., Fan, W., Marks, D.L. and Low, M.J. (2001). The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis Int J Obes Relat Metab Disord **25 Suppl 5**: S63-67.

Cowley, M.A., Smith, R.G., Diano, S., Tschöp, M., Pronchuk, N., Grove, K.L., Strasburger, C.J., Bidlingmaier, M., Esterman, M., Heiman, M.L., Garcia-Segura, L.M., Nillni, E.A., Mendez, P., Low, M.J., Sotonyi, P., Friedman, J.M., Liu, H., Pinto, S., Colmers, W.F., Cone, R.D., Horvath, T.L. (2003). The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis Neuron **37:** 649-661.

Cummings, D.E., Clement, K., Purnell, J.Q., Vaisse, C., Foster, K.E., Frayo, R.S., Schwartz, M.W., Basdevant, A. and Weigle, D.S. (2002). Elevated plasma ghrelin levels in Prader-Willi syndrome Nature Medicine **8**(7): 643-644.

Cummings, D.E., Frayo, R.S., Marmonier, C., Aubert, R. and Chapelot, D. (2004). Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues American Journal of Physiology-Endocrinology and Metabolism **287**(2): E297-E304.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E. and Weigle, D.S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans Diabetes **50**(8): 1714-1719.

Davis, C. and Woodside, D.B. (2002). Sensitivity to the rewarding effects of food and exercise in the eating disorders Comprehensive Psychiatry **43**(3): 189-194.

Davis, K.W., Wellman, P.J. and Clifford, P.S. (2007). Augmented cocaine conditioned place preference in rats pretreated with systemic ghrelin Regulatory Peptides **140**(3): 148-152.

Dickson, S.L., Hrabovszky, E., Hansson, C., Jerlhag, E., Alvarez-Crespo, M., Skibicka, K.P., Molnar, C.S., Liposits, Z., Engel, J.A. and Egecioglu, E. (2010). Blockade of central nicotine

acetylcholine receptor signaling attenuate ghrelin-induced food intake in rodents Neuroscience **171**(4): 1180-1186.

Dickson, S.L., Leng, G. and Robinson, I.C.A.F. (1993). Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurons Neuroscience **53**(2): 303-306.

Dickson, S.L. and Luckman, S.M. (1997). Induction of c-fos messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6 Endocrinology **138**(2): 771-777.

Disse, E., Bussier, A.L., Veyrat-Durebex, C., Deblon, N., Pfluger, P.T., Tschop, M.H., Laville, M. and Rohner-Jeanrenaud, F. (2010). Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content Physiology & Behavior **101**(2): 277-281.

Dornonville de la Cour, C., Lindqvist, A., Egecioglu, E., Tung, Y.C., Surve, V., Ohlsson, C., Jansson, J.O., Erlanson-Albertsson, C., Dickson, S.L. and Hakanson, R. (2005). Ghrelin treatment reverses the reduction in weight gain and body fat in gastrectomised mice Gut **54**(7): 907-913.

Druce, M.R., Neary, N.M., Small, C.J., Milton, J., Monteiro, M., Patterson, M., Ghatei, M.A. and Bloom, S.R. (2006). Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers International Journal of Obesity **30**(2): 293-296.

Dyr, W., Mcbride, W.J., Lumeng, L., Li, T.K. and Murphy, J.M. (1993). Effects of D1 and D2 dopamine receptor agents on ethanol-consumption in the high-alcohol-drinking (Had) line of rats Alcohol **10**(3): 207-212.

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K.P., Haage, D., Bohlooly, Y.M., Andersson, D., Bjursell, M., Perrissoud, D., Engel, J.A. and Dickson, S.L. (2010). Ghrelin increases intake of rewarding food in rodents Addict Biol **15**(3): 304-311.

Egecioglu, E., Skibicka, K.P., Hansson, C., Alvarez-Crespo, M., Friberg, A., Jerlhag, E., Engel, J.A. and Dickson, S.L. (2011). Hedonic and incentive signals for body weight control Reviews in Endocrine and Metabolic Disorders (in press).

El-Ghundi, M., George, S.R., Drago, J., Fletcher, P.J., Fan, T., Nguyen, T., Liu, C., Sibley, D.R., Westphal, H. and O'Dowd, B.F. (1998). Disruption of dopamine D-1 receptor gene expression attenuates alcohol-seeking behavior European Journal of Pharmacology **353**(2-3): 149-158.

Engel, J.A., Fahlke, C., Hulthe, P., Hard, E., Johannessen, K., Snape, B. and Svensson, L. (1988). Biochemical and behavioral evidence for an interaction between ethanol and calcium-channel antagonists Alcohol and Alcoholism **23**(3): A13-A13.

Erdie-Lalena, C.R., Holm, V.A., Kelly, P.C., Frayo, R.S. and Cummings, D.E. (2006). Ghrelin levels in young children with Prader-Willi syndrome Journal of Pediatrics **149**(2): 199-204.

Ericson, M., Löf, E., Stomberg, R. and Soderpalm, B. (2009). The smoking cessation medication varenicline attenuates alcohol and nicotine interactions in the rat mesolimbic dopamine system J Pharmacol Exp Ther **329**(1): 225-230.

Esler, W.P., Rudolph, J., Claus, T.H., Tang, W.F., Barucci, N., Brown, S.E., Bullock, W., Daly, M., DeCarr, L., Li, Y.X., Milardo, L., Molstad, D., Zhu, J., Gardell, S.J., Livingston, J.N. and Sweet, L.J. (2007). Small-molecule ghrelin receptor antagonists improve glucose tolerance, suppress appetite, and promote weight loss Endocrinology **148**(11): 5175-5185.

Faulconbridge, L.F., Cummings, D.E., Kaplan, J.M. and Grill, H.J. (2003). Hyperphagic effects of brainstem ghrelin administration Diabetes **52**(9): 2260-2265.

Grigson, P.S. (2002). Like drugs for chocolate: separate rewards modulated by common mechanisms?" Physiology & Behavior **76**(3): 345-346.

Gualillo, O., Caminos, J.E., Nogueiras, R., Seoane, L.M., Arvat, E., Ghigo, E., Casanueva, F.F. and Dieguez, C. (2002). Effect of food restriction on ghrelin in normal-cycling female rats and in pregnancy Obesity Research **10**(7): 682-687.

Guan, X.M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J.S., Smith, R.G., VanderPloeg, L.H.T. and Howard, A.D. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues Molecular Brain Research **48**(1): 23-29.

Gueorguiev, M., Korbonits, M., O'Grady, E., Lecoeur, C., Grossman, A.B. and Froguel, P. (2002). Mutation in the ghrelin gene associated with increase in body weight and reduction of insulin secretion in tall, obese children Diabetologia **45**: A176-A176.

Hansen, S., Harthon, C., Wallin, E., Lofberg, L. and Svensson, K. (1991). Mesotelencephalic dopamine system and reproductive behavior in the female rat: effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness Behav Neurosci **105**(4): 588-598.

Hernandez, L. and Hoebel, B.G. (1988). Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens Physiol Behav **44**(4-5): 599-606.

Hewson, A.K., Tung, L.Y.C., Connell, D.W., Tookman, L. and Dickson, S.L. (2002). The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic Diabetes **51**(12): 3412-3419.

Hillemacher, T., Kraus, T., Rauh, J., Weiss, J., Schanze, A., Frieling, H., Wilhelm, J., Heberlein, A., Groschl, M., Sperling, W., Kornhuber, J. and Bleich, S. (2007). Role of appetite-regulating peptides in alcohol craving: an analysis in respect to subtypes and different consumption patterns in alcoholism Alcohol Clin Exp Res **31**(6): 950-954.

Holden, C. (2001). Compulsive behaviors: Behavioral' addictions: Do they exist?" Science **294**(5544): 980-982.

Holst, B., Cygankiewicz, A., Jensen, T.H., Ankersen, M. and Schwartz, T.W. (2003). High constitutive signaling of the ghrelin receptor - Identification of a potent inverse agonist Molecular Endocrinology **17**(11): 2201-2210.

Holst, B. and Schwartz, T.W. (2004). Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation Trends in Pharmacological Sciences **25**(3): 113-117.

Holst, B. and Schwartz, T.W. (2006). Ghrelin receptor mutations--too little height and too much hunger J Clin Invest **116**(3): 637-641.

Howard, A.D., Feighner, S.D., Cully, D.F., Arena, J.P., Liberator, P.A., Rosenblum, C.I., Hamelin, M., Hreniuk, D.L., Palyha, O.C., Anderson, J., Paress, P.S., Diaz, C., Chou, M., Liu, K.K., McKee, K.K., Pong, S.S., Chaung, L.Y., Elbrecht, A., Dashkevicz, M., Heavens, R., Rigby, M., Sirinathsinghji, D.J.S., Dean, D.C., Melillo, D.G., Patchett, A.A., Nargund, R., Griffin, P.R., DeMartino, J.A., Gupta, S.K., Schaeffer, J.M., Smith, R.G. and VanderPloeg, L.H.T. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release Science **273**(5277): 974-977.

Jerlhag, E. (2008). Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine Addiction Biology **13**(3-4): 358-363.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Andersson, M., Svensson, L. and Engel, J.A. (2006a). Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward Addiction Biology **11**(1): 45-54.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Douhan, A., Svensson, L. and Engel, J.A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens Addiction Biology **12**(1): 6-16.

Jerlhag, E., Egecioglu, E., Dickson, S.L. and Engel, J.A. (2010a). Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference Psychopharmacology (Berl) **211**(4): 415-422.

Jerlhag, E., Egecioglu, E., Dickson, S.L. and Engel, J.A. (2011). Glutamatergic regulation of ghrelin-induced activation of the mesolimbic dopamine system Addict Biol **16**(1): 82-91.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Svensson, L. and Engel, J.A. (2008). Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors are involved in mediating the

ghrelin-induced locomotor stimulation and dopamine overflow in nucleus accumbens European Neuropsychopharmacolology **18**(7): 508-518.

Jerlhag, E., Egecioglu, E., Landgren, S., Salome, N., Heilig, M., Moechars, D., Datta, R., Perrissoud, D., Dickson, S.L. and Engel, J.A. (2009). Requirement of central ghrelin signaling for alcohol reward Proceedings of the National Academy of Sciences of the United States of America **106**(27): 11318-11323.

Jerlhag, E., Grotli, M., Luthman, K., Svensson, L. and Engel, J.A. (2006b). Role of the subunit composition of central nicotinic acetylcholine receptors for the stimulatory and dopamine-enhancing effects of ethanol Alcohol Alcohol **41**(5): 486-493.

Jerlhag, E., Landgren, S., Egecioglu, E., Dickson, S.L. and Engel, J.A. (2010b). The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice Alcohol (in press).

Jiang, H., Betancourt, L. and Smith, R.G. (2006). Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers Molecular Endocrinology **20**(8): 1772-1785.

Kaur, S. and Ryabinin, A.E. (2010). Ghrelin receptor antagonism decreases alcohol consumption and activation of perioculomotor urocortin-containing neurons Alcohol Clin Exp Res **34**(9): 1525-1534.

Kawahara, Y., Kawhara, H., Kaneko, F., Yamada, M., Nishi, Y., Tanaka, E. and Nishi, A. (2009). Peripherally administered ghrelin induces bimodal effects on mesolimbic dopamine system depending on food-consumptive states Journal of Pharmacological Sciences **109**: 187P-187P.

Keen-Rhinehart, E. and Bartness, T.J. (2005a). Fasting- and ghrelin-induced stimulation of food hoarding and food intake is attenuated by central NPYY1 receptor antagonist injection Hormones and Behavior **48**(1): 108-109.

Keen-Rhinehart, K. and Bartness, T.J. (2005b). Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters American Journal of Physiology-Regulatory Integrative and Comparative Physiology **288**(3): R716-R722.

Kim, D.J., Yoon, S.J., Choi, B., Kim, T.S., Woo, Y.S., Kim, W., Myrick, H., Peterson, B.S., Bin Choi, Y., Kim, Y.K. and Jeong, J. (2005). Increased fasting plasma ghrelin levels during alcohol abstinence Alcohol and Alcoholism **40**(1): 76-79.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach Nature **402**(6762): 656-660.

Korbonits, M., Gueorguiev, M., O'Grady, E., Lecoeur, C., Swan, D.C., Mein, C.A., Weill, J., Grossman, A.B. and Froguel, P. (2002). A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children Journal of Clinical Endocrinology and Metabolism **87**(8): 4005-4008.

Kraus, T., Schanze, A., Bayerlein, K., Hillemacher, T., Reulbach, U., Groschl, M., Kornhuber, J. and Bleich, S. (2005). Ghrelin levels are increased in alcoholism Pharmacopsychiatry **38**(5): 257-257.

Lall, S., Tung, L.Y.C., Ohlsson, C., Jansson, J.O. and Dickson, S.L. (2001). Growth hormone (GH)-independent stimulation of adiposity by GH secretagogues Biochemical and Biophysical Research Communications **280**(1): 132-138.

Landgren, S., Engel, J.A., Andersson, M.E., Gonzalez-Quintela, A., Campos, J., Nilsson, S., Zetterberg, H., Blennow, K. and Jerlhag, E. (2009). Association of nAChR gene haplotypes with heavy alcohol use and body mass Brain Res **1305 Suppl**: S72-79.

Landgren, S., Jerlhag, E., Hallman, J., Oreland, L., Lissner, L., Strandhagen, E., Thelle, D.S., Zetterberg, H., Blennow, K. and Engel, J.A. (2010). Genetic variation of the ghrelin signaling system in females with severe alcohol dependence Alcohol Clin Exp Res **34**(9): 1519-1524.

Landgren, S., Jerlhag, E., Zetterberg, H., Gonzalez-Quintela, A., Campos, J., Olofsson, U., Nilsson, S., Blennow, K. and Engel, J.A. (2008). Association of Pro-Ghrelin and GHS-R1A Gene Polymorphisms and Haplotypes With Heavy Alcohol Use and Body Mass Alcoholism-Clinical and Experimental Research **32**(12): 2054-2061.

Landgren, S., Simms, J.A., Hyytiä, P., Engel, J.A., Bartlett, S.E. and Jerlhag, E. (2011). Ghrelin receptor (GHS-R1A) antagonism suppresses both operant alcohol self-administration and high alcohol consumption in rats Addiction Biology (in press). Epub ahead of print. PMID: 21309944

Larsson, A., Edstrom, L., Svensson, L., Soderpalm, B. and Engel, J.A. (2005). Voluntary ethanol intake increases extracellular acetylcholine levels in the ventral tegmental area in the rat Alcohol and Alcoholism **40**(5): 349-358.

Larsson, A. and Engel, J.A. (2004). Neurochemical and behavioral studies on ethanol and nicotine interactions Neuroscience and Biobehavioral Reviews **27**(8): 713-720.

Leggio, L. (2010) Role of the ghrelin system in alcoholism: Acting on the growth hormone secretagogue receptor to treat alcohol-related diseases Drug News Perspect. **23(3)**:157-66.

Lindqvist, A., de la Cour, C.D., Stegmark, A., Hakanson, R. and Erlanson-Albertsson, C. (2005). Overeating of palatable food is associated with blunted leptin and ghrelin responses Regulatory Peptides **130**(3): 123-132.

Löf, E., Olausson, P., deBejczy, A., Stomberg, R., McIntosh, J.M., Taylor, J.R. and Soderpalm, B. (2007). Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and reinforcing properties of ethanol cues Psychopharmacology (Berl) **195**(3): 333-343.

Lyons, A.M., Lowery, E.G., Sparta, D.R. and Thiele, T.E. (2008). Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures Alcohol Clin Exp Res **32**(11): 1962-1968.

Malik, S., McGlone, F., Bedrossian, D., Dagher, A. (2008). Ghrelin modulates brain activity in areas that control appetitive behavior Cell Metabolism **7**(5): 400-409.

McKee, S.A., Harrison, E.L., O'Malley, S.S., Krishnan-Sarin, S., Shi, J., Tetrault, J.M., Picciotto, M.R., Petrakis, I.L., Estevez, N. and Balchunas, E. (2009). Varenicline reduces alcohol self-administration in heavy-drinking smokers Biol Psychiatry **66**(2): 185-190.

Mokrosinski, J. and Holst, B. (2010). Modulation of the constitutive activity of the ghrelin receptor by use of pharmacological tools and mutagenesis Methods Enzymol **484**: 53-73.

Naleid, A.M., Grace, M.K., Cummings, D.E. and Levine, A.S. (2005). Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens Peptides **26**(11): 2274-2279.

Olszewski, P.K., Grace, M.K., Billington, C.J. and Levine, A.S. (2003a). Hypothalamic paraventricular injections of ghrelin: effect on feeding and c-Fos immunoreactivity Peptides **24**(6): 919-923.

Olszewski, P.K., Li, D.H., Grace, M.K., Billington, C.J., Kotz, C.M. and Levine, A.S. (2003b). Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus Peptides **24**(4): 597-602.

Perello, M., Sakata, I., Birnbaum, S., Chuang, J.C., Osborne-Lawrence, S., Rovinsky, S.A., Woloszyn, J., Yanagisawa, M., Lutter, M. and Zigman, J.M. (2010). Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner Biological Psychiatry **67**(9): 880-886.

Potenza, M.N., Steinberg, M.A., Skudlarski, P., Fulbright, R.K., Lacadie, C.M., Wilber, M.K., Rounsaville, B.J., Gore, M.C. and Wexler, B.E. (2003). Gambling urges in pathological gambling - A functional magnetic resonance imaging study Archives of General Psychiatry **60**(8): 828-836.

Quarta, D., Di Francesco, C., Melotto, S., Mangiarini, L., Heidbreder, C. and Hedou, G. (2009). Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens Neurochemistry International **54**(2): 89-94.

Rada, P.V., Mark, G.P., Yeomans, J.J. and Hoebel, B.G. (2000). Acetylcholine release in ventral tegmental area by hypothalamic self-stimulation, eating, and drinking Pharmacology Biochemistry and Behavior **65**(3): 375-379.

Robinson, T.E. and Berridge, K.C. (1993). The neural basis of drug craving - an incentive-sensitization theory of addiction Brain Research Reviews **18**(3): 247-291.

Salomé, N., Haage, D., Perrissoud, D., Moulin, A., Demange, L., Egecioglu, E., Fehrentz, J.A., Martinez, J. and Dickson, S.L. (2009a). Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats Eur J Pharmacol **612**(1-3): 167-173.

Salomé, N., Hansson, C., Taube, M., Gustafsson-Ericson, L., Egecioglu, E., Karlsson-Lindahl, L., Fehrentz, J.A., Martinez, J., Perrissoud, D. and Dickson, S.L. (2009b). On the central mechanism underlying ghrelin's chronic pro-obesity effects in rats: new insights from studies exploiting a potent ghrelin receptor antagonist J Neuroendocrinol **21**(9): 777-785.

Schneider, E.R., Darby, R., Leibowitz, S.F. and Hoebel, B.G. (2007). Orexin, but not ghrelin, injected in the lateral hypothalamus increases alcohol intake in alcohol-drinking rats Alcoholism-Clinical and Experimental Research **31**(6): 199A-199A.

Schultz, W., Dayan, P. and Montague, P.R. (1997). A neural substrate of prediction and reward Science **275**(5306): 1593-1599.

Skibicka, K.P., Hansson, C., Alvarez-Crespo, M., Friberg, A. and Dickson, S.L. (2011b). Ghrelin directly targets the mesolimbic pathway to change food motivation in rats Neuroscience (in press).

Skibicka, K.P., Hansson, C., Egecioglu, E. and Dickson, S.L. (2011a). Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression Addiction Biology (in press). Epub ahead of print. PMID: 21309956.

Skibicka, K.P. and Dickson, S.L. (2011) Ghrelin and food reward, what's underneath: the story of potential underlying substrates. Peptides; Special Issue on ghrelin (in press).

Steensland, P., Simms, J.A., Holgate, J., Richards, J.K. and Bartlett, S.E. (2007). Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively

decreases ethanol consumption and seeking Proc Natl Acad Sci U S A **104**(30): 12518-12523.

Taber, M.T. and Fibiger, H.C. (1997). Feeding-evoked dopamine release in the nucleus accumbens: Regulation by glutamatergic mechanisms Neuroscience **76**(4): 1105-1112.

Tanaka, M., Naruo, T., Nagai, N., Kuroki, N., Shiiya, T., Nakazato, M., Matsukura, S. and Nozoe, S. (2003). Habitual binge/purge behavior influences circulating ghrelin levels in eating disorders Journal of Psychiatric Research **37**(1): 17-22.

Tang, D., Han, J.E., Rangel, A. and Dagher, A. (2011). Administration of ghrelin increases willingness to pay for food: A neuroeconomics approach to obesity. Meeting: Keystone Symposia on Molecular and Cellular Biology: Obesity, , Keystone resort, CO, USA.

Tessari, M., Catalano, A., Pellitteri, M., Di Francesco, C., Marini, F., Gerrard, P.A., Heidbreder, C.A. and Melotto, S. (2007). Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats Addiction Biology **12**(1): 22-29.

Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueiras, R., Perez-Tilve, D., Pfluger, P., Castaneda, T.R., Muzzin, P., Schurmann, A., Szanto, I., Tschop, M.H. and Rohner-Jeanrenaud, F. (2006). Ghrelin action in the brain controls adipocyte metabolism Journal of Clinical Investigation **116**(7): 1983-1993.

Toshinai, K., Date, Y., Murakami, N., Shimada, M., Mondal, M.S., Shimbara, T., Guan, J.L., Wang, Q.P., Funahashi, H., Sakurai, T., Shioda, S., Matsukura, S., Kangawa, K. and Nakazato, M. (2003). Ghrelin-induced food intake is mediated via the orexin pathway Endocrinology **144**(4): 1506-1512.

Tschöp, M., Smiley, D.L. and Heiman, M.L. (2000). Ghrelin induces adiposity in rodents Nature **407**(6806): 908-913.

Tschöp, M., Wawarta, R., Riepl, R.L., Friedrich, S., Bidlingmaier, M., Landgraf, R. and Folwaczny, C. (2001). Post-prandial decrease of circulating human ghrelin levels Journal of Endocrinological Investigation **24**(6): Rc19-Rc21.

Ukkola, O., Ravussin, E., Jacobson, P., Snyder, E.E., Chagnon, M., Sjostrom, L. and Bouchard, C. (2001). Mutations in the preproghrelin/ghrelin gene associated with obesity in humans Journal of Clinical Endocrinology and Metabolism **86**(8): 3996-3999.

Vartiainen, J., Kesaniemi, Y.A. and Ukkola, O. (2006). Sequencing analysis of ghrelin gene 5' flanking region: relations between the sequence variants, fasting plasma total ghrelin concentrations, and body mass index Metabolism **55**(10): 1420-1425.

Verhagen, L.A., Egecioglu, E., Luijendijk, M.C., Hillebrand, J.J., Adan, R.A. and Dickson, S.L. (2010). Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity Eur Neuropsychopharmacol.

Vivenza, D., Rapa, A., Castellino, N., Bellone, S., Petri, A., Vacca, G., Aimaretti, G., Broglio, F. and Bona, G. (2004). Ghrelin gene polymorphisms and ghrelin, insulin, IGF-I, leptin and anthropometric data in children and adolescents European Journal of Endocrinology **151**(1): 127-133.

Volkow, N.D., Fowler, J.S. and Wang, G.J. (2003). The addicted human brain: insights from imaging studies Journal of Clinical Investigation **111**(10): 1444-1451.

Volkow, N.D. and Li, T.K. (2004). Drug addiction: the neurobiology of behaviour gone awry Nat Rev Neurosci **5**(12): 963-970.

Volkow, N.D., Wang, G.J., Maynard, L., Jayne, M., Fowler, J.S., Zhu, W., Logan, J., Gatley, S.J., Ding, Y.S., Wong, C. and Pappas, N. (2003). Brain dopamine is associated with eating behaviors in humans International Journal of Eating Disorders **33**(2): 136-142.

Wang, H.J., Geller, F., Dempfle, A., Schauble, N., Friedel, S., Lichtner, P., Fontenla-Horro, F., Wudy, S., Hagemann, S., Gortner, L., Huse, K., Remschmidt, H., Bettecken, T., Meitinger, T., Schafer, H., Hebebrand, J. and Hinney, A. (2004). Ghrelin receptor gene: Identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature Journal of Clinical Endocrinology and Metabolism **89**(1): 157-162.

Wellman, P.J., Davis, K.W. and Nation, J.R. (2005). Augmentation of cocaine hyperactivity in rats by systemic ghrelin Regulatory Peptides **125**(1-3): 151-154.

Wise, R.A. and Bozarth, M.A. (1987). A psychomotor stimulant theory of addiction Psychological Review **94**(4): 469-492.

Wren, A.M., Seal, L.J., Cohen, M.A., Brynes, A.E., Frost, G.S., Murphy, K.G., Dhillo, W.S., Ghatei, M.A. and Bloom, S.R. (2001). Ghrelin enhances appetite and increases food intake in humans Journal of Clinical Endocrinology and Metabolism **86**(12): 5992-5995.

Wren, A.M., Small, C.J., Abbott, C.R., Dhillo, W.S., Seal, L.J., Cohen, M.A., Batterham, R.L., Taheri, S., Stanley, S.A., Ghatei, M.A. and Bloom, S.R. (2001). Ghrelin causes hyperphagia and obesity in rats Diabetes **50**(11): 2540-2547.

Wren, A.M., Small, C.J., Ward, H.L., Murphy, K.G., Dakin, C.L., Taheri, S., Kennedy, A.R., Roberts, G.H., Morgan, D.G.A., Ghatei, M.A. and Bloom, S.R. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion Endocrinology **141**(11): 4325-4328.

Wurst, F.M., Graf, I., Ehrenthal, H.D., Klein, S., Backhaus, J., Blank, S., Graf, M., Pridzun, L., Wiesbeck, G.A. and Junghanns, K. (2007). Gender differences for ghrelin levels in alcohol-dependent patients and differences between alcoholics and healthy controls Alcoholism-Clinical and Experimental Research **31**(12): 2006-2011.

Yoon, S.J., Pae, C.U., Lee, H., Choi, B., Kim, T.S., Lyoo, I.K., Kwon, D.H. and Kim, D.J. (2005). Ghrelin precursor gene polymorphism and methamphetamine dependence in the Korean population Neurosci Res **53**(4): 391-395.

Zigman, J.M., Jones, J.E., Lee, C.E., Saper, C.B. and Elmquist, J.K. (2006). Expression of ghrelin receptor mRNA in the rat and the mouse brain Journal of Comparative Neurology **494**(3): 528-548.

Zimmermann, U.S., Buchmann, A., Steffin, B., Dieterle, C. and Uhr, M. (2007). Alcohol administration acutely inhibits ghrelin secretion in an experiment involving psychosocial stress Addiction Biology **12**(1): 17-21.

Figure Legends

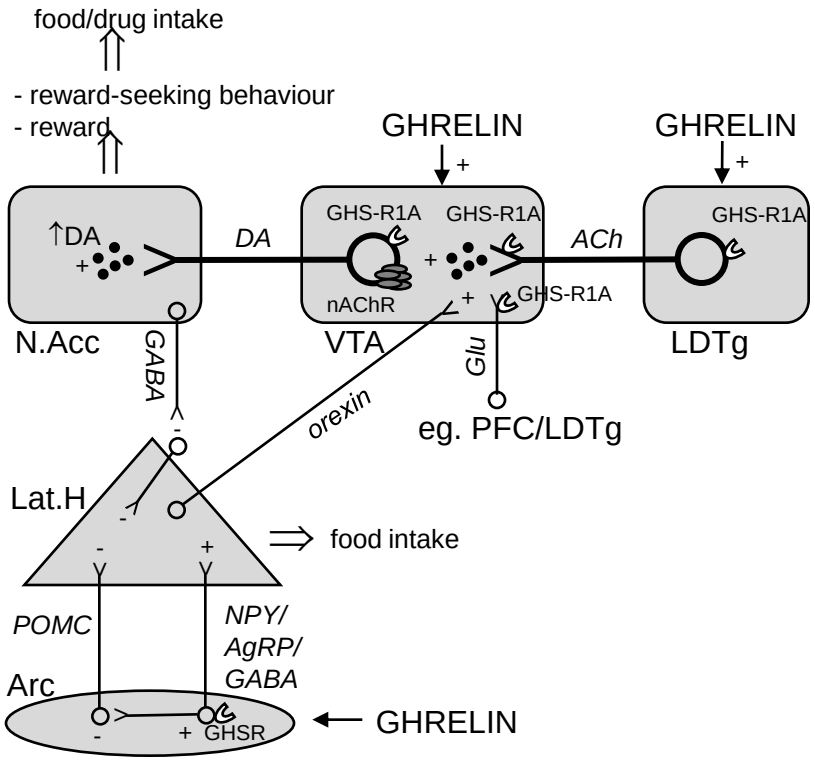
Figure 1. Schematic representation of key pathways through which ghrelin may regulate food intake and reward-seeking behaviour.

The central ghrelin signalling system is required for reward for natural reinforcers such as food as well as from addictive drugs, including alcohol. Ghrelin activates the cholinergic-dopaminergic reward link via pre- and/or post-synaptic GHS-R1A signalling in the VTA as well as the LDTg. By this route, the VTA dopamine neurons that project to the N.Acc become activated, either directly or involving cholinergic and glutamatergic afferents. Indeed, cholinergic neurons in LDTg appear to be a direct target of ghrelin, although cholinergic (and glutamatergic) afferents to the VTA dopamine cells could also arise in the PFC. Ghrelin's effects to enhance dopamine signalling causes reward and reward seeking-behaviour, which may lead to the intake of food (especially palatable food) and drugs of abuse. Ghrelin also increases food intake via activation of homeostatic feeding circuits in the Arc involving direct actions on orexigenic NPY neurons, that co-localise the anorexigenic peptide, AgRP, and signal to another anorexigenic POMC neurons via increased GABAergic signalling. The NPY-POMC circuits signal to other hypothalamic areas such as the Lat.H.; by activating this system, ghrelin may not only increase food intake but also increase motivated behaviour for foods and other reward reinforcers, involving recruitment of other pathways that include the orexin neurons of the Lat.H.

ACh, acetylcholine; AgRP, agouti-related peptide; DA, dopamine; GABA; gamma-aminobutyric acid; Glu, glutamate; NPY, neuropeptide Y; POMC, proopiomelanocortin;

LDTg, laterodorsal tegmental area; VTA, ventral tegmental area; N.Acc, nucleus accumbens, Lat.H, lateral hypothalamus; Arc, arcuate nucleus; GHS-R1A, growth hormone secretagogue receptor; nAChR, nicotinic ACh receptor.

Figure


food/drug intake
- reward-seeking behaviour
- reward
GHRELIN
+
GHRELIN
+
↑DA
+
DA
GHS-R1A
GHS-R1A
+
nAChR
+
GHS-R1A
ACh
GHS-R1A
N.Acc
GABA
-
VTA
Glu
LDTg
eg. PFC/LDTg
orexin
Lat.H
-
food intake
-
+
POMC
NPY/
AgRP/
GABA
Arc
-
+ GHSR
GHRELIN