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Vagal K_{ATP} channels are the key to ghrelin's orexigenic action

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Food intake is a highly regulated process which utilises humoral and neural communication to achieve balance of intake and expenditure. The vagus nerve is intimately involved in regulating food intake, particularly through afferent projections to the stomach and small intestine. The vagal afferent neurones, which comprise about 90% of the vagus, are highly responsive to a variety of stimuli such as mechanical perturbation and chemical exposure (Dockray, 2014). Some of the main regulators of vagal activity are peptides released from the epithelium of the gastrointestinal tract. Peptides such as cholecystokinin (CCK), ghrelin and leptin are expressed and released from discrete populations of gastrointestinal epithelial cells, which are normally located close to vagal afferent sensory endings (Kentish & Page, 2015). Whilst leptin and CCK are among a multitude of anorexigenic peptides released from the gastrointestinal tract, ghrelin is unique in that to date it is the only identified orexigenic peptide released from the periphery (Dockray, 2014).

However, there is much debate about the relative importance of peripheral *vs.* central effects of such peptides, including ghrelin, in regards to food intake. A central site of ghrelin action has been proposed and well characterised in terms of the ability of ghrelin to activate appetite promoting agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurones (Ferrini *et al.* 2009). This potentiating ability has been suggested to be due to activation of T-type or N-type Ca^{2+} channels through a protein kinase A (PKA) dependent mechanism (Ferrini *et al.* 2009). Additionally, mechanisms involving AMP-activated protein kinase (AMPK) and phospholipase C (PLC) have also been suggested to be involved (Ferrini *et al.* 2009). The net effect of ghrelin activation of these neurones is to cause the release of the neurotransmitters AgRP and NPY, and the inhibitory neurotransmitter GABA, which in addition to modulating neurones in other hypothalamic nuclei also suppresses activity in the adjacent pro-opiomelanocortin (POMC) neurones, which classically act to reduce appetite and food intake (Ferrini *et al.* 2009).

The vagal nerve, on the other hand, communicates using glutamate as its major neurotransmitter and excitation of vagal afferents has been demonstrated to have an anorexigenic effect (Dockray, 2014). Therefore, unlike its effects on AgRP/NPY neurones, if ghrelin is able to modulate food intake through a vagal pathway it would need to have an inhibitory effect. Previous studies have demonstrated ghrelin can modulate vagal nerves from the upper gastrointestinal tract including the oesophagus, stomach and jejunum, inhibiting the first two and augmenting the third (Kentish & Page, 2015). However, little had been established in regards to the cellular signalling mechanism by which ghrelin modulates vagal activity and thus may promote food intake.

In a recent paper in *The Journal of Physiology*, Grabauskas *et al.* (2015) examined the role of ATP-sensitive potassium (K_{ATP}) channels in the nodose ganglia in mediating the orexigenic effects of ghrelin. Through an elegant combination of *in vitro* and *in vivo* experimentation, Grabauskas *et al.* demonstrated ghrelin requires a vagal growth hormone secretagogue receptor 1a (GHSR-1a)–phosphoinositide 3-kinase (PI3K)–extracellular signal-regulated protein kinases 1/2 (ERK1/2)–K_{ATP} channel pathway to increase acute food intake by reducing vagal afferent activity. They began by establishing in isolated vagal neurones that ghrelin decreased excitability by inducing increased potassium currents through K_{ATP} channels. This was supported by co-localisation of the ghrelin receptor (GHS-R1a) and the Kir6.2 subunit of the K_{ATP} channel in nodose neurones. Through the incubation of cells with a comprehensive list of second messenger inhibitors, the authors concluded there was involvement of a Gαi/o protein and PI3K, but not PKA or PLC. These data provide an elegant mechanism for ghrelin's inhibitory action on vagal neurones, which is distinct from the central pathway. Finally, the authors showed that *in vivo* electroporation of siRNA against Kir6.2 into the right nodose ganglia resulted in significant attenuation of the food intake stimulatory effect of ghrelin, which suggested that the vagal nerve was integral in the orexigenic effect of ghrelin. The paper by Grabauskas *et al.* raises a number of discussion points some of which are addressed below.

The paradigm suggested by Grabauskas *et al.* is that ghrelin is likely to be acting directly on the soma to drive food intake. This is based on a single report that found ghrelin still increased food intake after a subdiaphragmatic vagal deafferentation. However, this is an isolated finding with others finding vagotomies ablate the orexigenic effect of ghrelin (reviewed in Kentish & Page, 2015) suggesting the actual vagal projections to the viscera may still be important in the orexigenic effect of ghrelin. It would have been interesting to see if the *in vivo* recordings and feeding studies had been repeated in vagotomised rats whether the vagal modulatory role of ghrelin and food intake promoting effect were still observed or not. Such studies would provide much stronger evidence either supporting their hypothesis of a direct effect on the soma or suggesting modulation at the endings as the *in vivo* site of action.

If ghrelin is acting solely at the level of the soma, the physiological relevance of the findings needs to be more closely considered. The concentration of ghrelin applied extracellularly in the patch clamp experiments, 30 nM, and the I.V./I.P. injection (assuming an equal concentration through the circulation of a 200 g rat), ~110 nM, far exceed circulating levels in rats, which tend to be around < 1 nM. Given the close apposition of ghrelin cells in the stomach and vagal endings, such concentrations may be seen locally at the level of the endings before the concentration is diluted down in the systemic circulation. Thus, to confirm the feasibility of the proposed mechanism, it needs to be determined whether systemic physiological levels of ghrelin are capable of inducing the potassium currents and neuronal hyperpolarization that are proposed to at least partially mediate the orexigenic effect of ghrelin reported by Grabauskas *et al.*

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The identification of the effector channel activated by ghrelin is perhaps the most important finding of this paper. Given the role of K_{ATP} channels in linking energy homeostasis with neural excitability, this makes it a logical effector channel for ghrelin, as when ghrelin levels are high, such as during a period of fasting, glucose is usually low increasing the channel opening probability. Conversely, when glucose is high, such as after feeding, ghrelin is low, thus allowing the channels to switch to closed configuration. By utilising two signals which are usually not high at the same time (glucose and ghrelin) there can be strong modulation of a system, just like in a car when you release the brake and push the accelerator. An interesting factor is that leptin, considered the opposing force to ghrelin, has been documented to activate K_{ATP} channels in a variety of neurones (Mirshamsi *et al.* 2004). There are leptin receptors in the nodose ganglia and leptin has been shown to signal through PI3K in vagal afferents, but not activate K_{ATP} channels. Thus, K_{ATP} channels are activated in a highly neuronal specific manner, by a variety of conflicting substances.

An interesting result that was not discussed is that the K_{ATP} channel antagonist tolbutamide reduced outward current in isolated nodose neurones independent of the addition of ghrelin. In addition knockdown of Kir6.2 in the absence of ghrelin *in vivo* resulted in significantly reduced acute food intake. It is well established that GHS-R1a possesses an unusually high level of constitutive activity (~70%), which may explain this result. This attribute of GHS-R1a is being exploited by pharmaceutical companies, developing inverse agonists to 'switch off' this high level of ghrelin independent activity. In relation to the paper by Grabauskas *et al.*, it would be interesting to determine what the relative effect of a GHS-R1a inverse agonist would be in order to determine whether all the K_{ATP} activity which is lost by tolbutamide incubation or Kir6.2 siRNA was being caused by GHS-R1a constitutive activity or another pathway which modulates K_{ATP} channel activity.

The paper by Grabauskas *et al.* strengthens the importance of the vagus nerve for ghrelin induced food intake. The identification of the specific signalling cascade used by peptides such as ghrelin on the vagus nerve provides unique opportunities to develop agents to modulate food intake via peripheral means, which could potentially be used to treat food intake disorders such as obesity or cachexia without the central side-effects which have stymied previous pharmaceutical treatments.

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Additional information

Competing interests

The author declares no competing financial interests.

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