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Integrative capacity of the caudal brainstem in the control of food intake

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The caudal brainstem nucleus of the solitary tract (NTS) is the initial central nervous system (CNS) terminus for a variety of gastrointestinal mechanical, nutrient chemical and gut peptide signals that limit the amount of food consumed during a meal. It receives neuroanatomical projections from gut vagal and non-vagal visceral afferents that mediate the CNS representation of these meal-stimulated gut feedback signals, and is reciprocally connected to a range of hypothalamic and limbic system sites that play significant roles in the neural processing of meal-related stimuli and in determining food consumption. Neurons in the NTS also contains elements of leptinergic and melanocortinergic signalling systems, presenting the possibility that the brainstem actions of these neuropeptides affect both the NTS processing of meal-stimulated gut afferent neural activity and its behavioural potency. Taken together, these features suggest that the NTS is ideally situated to integrate central and peripheral signals that determine meal size. This manuscript will review recent support from molecular genetic, neurophysiological and immunocytochemical studies that begin to identify and characterize the types of integrative functions performed within the NTS, and highlight the extent to which they are consistent with a causal role for NTS integration of peripheral gut and central neuropeptide signals important in the control of food intake.

Keywords: vagus; gut–brain communication; visceral afferent; ingestive behaviour

1. INTRODUCTION

The nucleus of the solitary tract (NTS) in the caudal brainstem of the rat is the primary neuroanatomical site receiving visceral afferent information from postoral regions of the alimentary tract critical for the negative-feedback control of food intake. The majority of published data demonstrate that the transmission of meal-related visceral afferent feedback signals to the brainstem is mediated by the afferent vagus nerve, which terminates in the NTS. Interpretations of results from these studies rely in part on the examination of the behavioural or neurophysiological consequences of stimulation of the gut vagus. Selective mechanical, chemical and gut peptidergic stimulation of gastric and duodenal compartments of the gastrointestinal tract result in dose-dependent reductions in subsequent food intake and meal size (e.g. Schwartz 2000; Peters *et al.* 2005*a*,*b*). Furthermore, subdiaphragmatic afferent vagal fibres supplying the upper gastrointestinal tract are dose-dependently activated by a range of gastroduodenal mechanical and chemical properties of foods, including distension, nutrient chemical composition, pH and osmolarity (see Schwartz (2000) for review). Support for a role for these signals in the negative-feedback control of ingestion comes from studies involving selective surgical, chemical or genetically mediated interruption of gut vagal afferent traffic (e.g. Smith *et al.* 1985; Schwartz *et al.* 1999; Fox *et al.* 2001). This interruption prevents neural food-stimulated signals from reaching their brainstem targets, with the result that meal size after gut afferent vagotomy may be chronically increased, although body weight does not exceed that of surgical controls (e.g. Schwartz *et al.* 1999).

Recent work of Sclafani *et al.* (2003) also implicates gut non-vagal splanchnic afferent fibres in this negative-feedback control of ingestion following intestinal nutrient infusions. Importantly, splanchnic afferents from the gut also project to the NTS via the spinosolitary tract (Menetrey & Basbaum 1987; Menetrey & De Pommery 1991), supporting a convergence of vagal and non-vagal meal-stimulated negative-feedback signals in the NTS.

Extensive studies, primarily those from Grill and colleagues, have revealed the remarkable regulatory capabilities of the rat caudal brainstem, in terms of maintained sensitivity to the feeding modulatory effects of gut satiety peptides and a range of gastrointestinal meal-related signals (Grill & Kaplan 2002). The results of these studies demonstrate the sufficiency of this neural substrate to receive and respond appropriately to neurohumoural negative-feedback signals from the periphery in limiting food intake. This work, however, does not address the neuronal response properties underlying the regulatory capacity of the caudal brainstem, nor the ways in which the caudal brainstem functions in the intact neuraxis, where reciprocal neuroanatomical connections between forebrain limbic and hypothalamic structures important in the control of food intake have been demonstrated. The present article will focus on the identification and characterization of the ways in which neurons in the NTS

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respond to multiple humoural and gastrointestinal neural signals important in the negative-feedback control of food intake. Specifically, a striking feature of gut-sensitive NTS neurons is their capacity to integrate signals arising from multiple peripheral and central neurohumoural sources, in ways consistent with a causal role for this integration in the control of food intake. The greatest experimental progress thus far comes from studies examining the role of leptinergic and melanocortinergic modulation of NTS neuronal function in feeding. Consequently, this monograph will centre on the influence of these two systems in the NTS.

2. CHARACTERIZATION OF GUT INPUT TO THE NUCLEUS OF THE SOLITARY TRACT

Critical to any potential role for nucleus of the solitary tract (NTS) neuronal responses in the negative-feedback control of food intake is their fidelity to the neural and behavioural impact of meal-related signals from the gut. Single afferent fibres with receptive fields in the gastrointestinal tract are dose-dependently responsive to increased gastric volume (Mathis *et al.* 1998), small intestinal nutrient concentration (Schwartz *et al.* 1998) and exogenous administration of the gut–brain peptide cholecystokinin (Schwartz 2000), and increases in each of these stimuli dose-dependently reduce meal size. These stimuli have also been demonstrated to produce increases in the number of NTS neurons activated as measured by expression of the neuronal protein c-fos (Rinaman *et al.* 1998; Zittel *et al.* 1994; Monnikes *et al.* 1997; Phifer & Berthoud 1998; Berthoud *et al.* 2001). In only one of these studies the dose-dependent effects were evaluated (Rinaman *et al.* 1998), so the extent to which increased number of c-fos labelled cells may map onto decreases in food intake remains largely unclear. However, in neurophysiological studies, both gastric loads and peripheral cholecystonin (CCK) have been shown to dose-dependently increase the single unit NTS spike rate, across ranges that in feeding studies dose-dependently reduce food intake (Yuan *et al.* 2000; Schwartz & Moran 2002). These data provide evidence of good fidelity between the response properties of individual gut-recipient neurons and feeding inhibition.

Subdiaphragmatic vagal afferents also provide integrated negative-feedback signals from the gut to the NTS. In neurophysiological studies of single vagal afferents supplying the stomach, Schwartz *et al.* (1993) have shown that combinations of CCK and gastric loads produce greater spike rates than either stimulus alone, and that subthreshold gastric loads combined with subthreshold doses of CCK elevate neurophysiological spike rate when co-administered. Peripheral application of leptin increases the subsequent gut vagal afferent neural response to CCK (Wang *et al.* 1997, 1998), and, conversely, CCK appears to gate the response to peripheral leptin (Wang *et al.* 1997, 1998, 2000; Peters *et al.* 2005*a*,*b*). Thus, at the level of peripheral gut-sensitive vagal afferents, there is direct neurophysiological evidence of integration of multiple modalities of peripheral stimuli, including gut mechanical distension, gut peptides and adiposity hormones. Such integrative properties are consistent with the feeding inhibitory consequences of these stimulus combinations. Co-administration of exogenous CCK analogues and gastric loads are more effective in reducing meal size than either stimulus alone (Schwartz *et al.* 1991), and combinations of peripheral leptin and CCK also produce greater reductions in feeding than either peptide alone (Peters *et al.* 2005*a*). It might be expected that these integrative properties, resulting in increased peripheral vagal activation, would be represented by increased activation of gut-sensitive NTS neurons, either in terms of an increased number of c-fos expressing neurons and/or an increased neurophysiological response, but neither measure has been evaluated for these stimulus combinations, and this remains an important open question.

3. INTEGRATION WITHIN THE NUCLEUS OF THE SOLITARY TRACT—LEPTIN

When it has been possible to document the integrative capacity of the nucleus of the solitary tract (NTS), multiple forms of integration can be characterized, some of which rely on forebrain inputs, while others appear to originate in the NTS itself. NTS neurons receive descending projections from a variety of hypothalamic and limbic sites implicated in the control of ingestion, including the lateral and paraventricular hypothalamic nuclei, and the central nucleus of the amygdala (van der Kooy *et al.* 1984; Zhang *et al.* 1999, 2003; Jiang *et al.* 2003). Neurophysiological studies of Zhang *et al.* (1999, 2003) and Zhang & Fogel (2002) comprise a number of demonstrations that electrical or neurochemical glutaminergic stimulation of these sites are sufficient to inhibit the increase in spike rate in single NTS neurons produced by gastroduodenal distension. Functional roles for these neuromodulatory influences on feeding have not been determined, but given the reciprocal relationship between increased gut-sensitive NTS activity and decreased food intake, amygdalar or hypothalamic stimulation could be predicted to promote increased feeding.

In rats with pyloric cuffs that block the flow of nutrients from the stomach to the intestine, combined gastric loads and duodenal nutrient infusions elicit significantly greater numbers of c-fos labelled NTS neurons than either gastric or duodenal infusions alone (Emond *et al.* 2001*a*). This increase suggests that the NTS has an additive representation of gastroduodenal stimuli from distinct gastric and duodenal compartments that arises *within* the NTS. Consistent with this behavioural finding is neurophysiological evidence demonstrating that there are distinct subpopulations of neurons in different subnuclei of the NTS that have differential response properties to gastric and duodenal distension. Zhang *et al.* (1995) have shown that neurons in the gelatinous subnucleus are sensitive to gastric but not duodenal distension, that neurons in the subpostremal region respond to duodenal but not gastric distension, and medial subnucleus are excited by both gastric and duodenal distension. From the perspective of characterizing the integrative properties of gut-sensitive NTS neurons relevant to the control of food intake, it would be important to determine

whether and to what degree these combinations of stimuli produced changes in the spike activity of individual gut-recipient NTS neurons different from those elicited by either stimulus alone.

The integrative capacity of the NTS also extends to the co-processing of centrally active humoural adiposity signals, such as leptin, with peripheral signals that are generated by the gastrointestinal presence of nutrients within a meal. Exogenous administration of leptin reduces food intake by reducing meal size, without affecting meal frequency (Eckel *et al.* 1998; Kahler *et al.* 1998). This is important because it permits the testable suggestion that leptin acts by modulating the feeding inhibitory potency of gut negative-feedback signals arising from ingested food during a meal. Consistent with this suggestion are data demonstrating that fasting reduces plasma leptin and CCK satiety, and intravenous replacement of leptin in fasted animals at doses targeted to achieve non-fasting levels restored sensitivity to the feeding inhibitory effects of peripheral CCK (McMinn *et al.* 2000).

Central forebrain ventricular leptin administration dose-dependently increases the feeding inhibitory potency of two meal-related stimuli that limit meal size: CCK and gastric loads (Barrachina *et al.* 1997; Emond *et al.* 1999, 2001*a*,*b*; Wang *et al.* 2000). Central leptin at doses that have no effect on feeding when administered alone become effective when combined with gastric loads or CCK. Because leptin was administered centrally in these studies, the data suggested that the effectiveness of the combination of these stimuli arose from changes in the way the central nervous system (CNS) represents peripheral feedback signals from the gut.

Consistent with this suggestion, subsequent studies found that identical combinations of stimuli increased the number of labelled NTS neurons (Emond *et al.* 1999, 2001*a*,*b*). Central leptin alone was ineffective in stimulating NTS c-fos expression, but leptin administration in the presence of CCK or gastric loads increased the number of c-fos expressing NTS neurons beyond that produced by either CCK or gastric load alone. The rostrocaudal localization of the greatest c-fos activation was along the medial extent of the NTS, where the majority of gut vagal afferent terminations lie in the medial and commissural subnuclei. Neurophysiological studies of single gut-recipient NTS neurons demonstrate that central leptin increases their excitatory response to gastric loads across a physiological range of gastric volumes (Schwartz & Moran 2002). These data demonstrate that gut vagal afferent negative-feedback signals and central adiposity signals are integrated at the level of the individual neuron in ways consistent with the increased effectiveness of combinations of central leptin and peripheral gastric loads in the reduction of food intake. Taken together, these data show that the NTS integrates central adiposity signals with peripheral meal-related negative-feedback signals by: (i) recruiting the activation of a larger population of neurons, and (ii) increasing the single neuron neurophysiological response to stimulus combinations above levels of activity produced by either alone.

The best evidence for molecular mediators of the brainstem integration of central leptin and peripheral meal-related gut negative-feedback signals in the control of food intake comes from recent work evaluating the role of descending paraventricular nucleus (PVN) oxytocinergic input to the NTS. Work of Schwartz and colleagues has shown that (i) PVN neurons activated by third ventricular leptin project to the NTS, (ii) oxytocin receptor antagonist administration into the fourth, but not third ventricle stimulates feeding (Blevins *et al.* 2004), and (iii) oxytocin receptor antagonists block the effect of leptin and the synergistic inhibitory effects of combined CCK and central leptin. The descending leptinergic modulation of CCK-induced brainstem neuronal activation is mediated in part by oxytocinergic terminals, as: (i) oxytocinergic immunoreactive fibres are in close apposition to CCK-activated NTS neurons (Blevins *et al.* 2003), and (ii) third intracerebroventricular application of the oxytocin receptor antagonist OVT attenuates the potentiating effect of forebrain leptin in CCK-induced NTS c-fos expression (Blevins *et al.* 2004).

Support for a physiological role for brainstem NTS integration of CCK and leptin signals in the control of food intake also comes from recent work of Morton *et al.* (2003), who evaluated the eating behaviour of *fak*/*fak* Koletsky rats that genetically lack leptin receptors. These animals are hyperphagic relative to their +/+ leptin competent littermates, and their overeating is manifested by increased meal size, with no change in meal frequency. They found that in tests of solid chow intake, Koletstky rats were also less sensitive to peripheral feeding inhibitory doses of CCK, and in the absence of food, CCK elicited less brainstem c-fos expression than in +/+ controls. Adenoviral replacement of the functional leptin receptor LeprB directed at the hypothalamic arcuate nucleus resulted in: (i) reduced food intake via reduced meal size, (ii) increased behavioural sensitivity to peripheral CCK, and (iii) increased numbers of c-fos expressing neurons in the NTS compared to fak/fak rats treated with adenoviral control injections (Morton *et al.* 2005).

The specific molecular events and inter-NTS circuitry mediating the increase in NTS neuronal activation in response to combinations of leptin and CCK or gastric load stimuli are not known, but roles of leptin and glutamate receptors in the NTS have been suggested. The functional ObRb form of the leptin receptor linked to the JAK–STAT transduction pathway has been identified in NTS neurons (Grill *et al.* 2002; Hosoi *et al.* 2002), and parenchymal administration of leptin into the dorsal vagal complex, including the NTS, has been shown to reduce food intake (Grill *et al.* 2002). It remains an important open question, whether local brainstem administration of leptin will potentiate the feeding inhibitory or NTS neuronal activation effects of meal-related gut negative-feedback stimuli. Brainstem administration of glutamatergic *N*-methyl-D-aspartate (NMDA) antagonists blocks CCK-induced reductions in food intake (Covasa *et al.* 2004), and delay meal termination (Treece *et al.* 1998). Furthermore, glutamate receptors are present on gut-recipient NTS neurons activated by at least two meal-related stimuli,

gastric distension and duodenal nutrient administration (Berthoud *et al.* 2001), but it is not known whether these are interneurons or NTS neurons that directly receive primary vagal afferent input. Surprisingly, brainstem administration of the NMDA receptor antagonist MK801: (i) failed to increase food intake following gastric loading confined to the stomach (Covasa *et al.* 2000*a*), and (ii) failed to block the inhibition of sham feeding produced by intestinal nutrient infusions (Covasa *et al.* 2000*b*). These apparently discrepant results suggest a more complex circuit within the NTS mediating the relationship between glutamatergic transmission of gut negative-feedback signals and the control of food intake.

The ability of the NTS to integrate peripheral meal-related signals with central leptin has recently been extended to include the feeding inhibitory peptide bombesin (BN). Ladenheim *et al.* (2005) has shown that peripheral administration of BN doses below those required to reduce feeding also become effective when administered in conjunction with central administration of subthreshold doses of leptin. Furthermore, leptin dose-dependently amplifies the feeding inhibitory effects of sub- and suprathreshold feeding inhibitory doses of BN. These combinations of BN and leptin also produce greater numbers of NTS neurons expressing c-fos than that elicited by either peptide alone.

4. INTEGRATION WITHIN THE NUCLEUS OF THE SOLITARY TRACT—MELANOCORTINS

Central administration of the melanocortin-3/4 receptor agonist melanotan II (MTII) reduces food intake by reducing meal size (Azzara *et al.* 2002; Williams *et al.* 2002), and fourth ventricular application is sufficient to achieve these reductions (Williams *et al.* 2000; Zheng *et al.* 2005). Pro-opiomelanocortin (POMC) neurons exist within the brainstem, and hypothalamic arcuate POMC neurons project to the dorsal vagal complex (Zheng *et al.* 2005; Ellacott *et al.* 2006). Furthermore, alpha melanocyte stimulating hormone containing immunoreactive fibres are in close neuroanatomical proximity to nucleus of the solitary tract (NTS) neurons activated by gastric distension, suggesting a neural circuit that could permit the physiological melanocortinergic modulation of ascending gut meal-related negative-feedback signals. Consistent with this suggestion, Fan *et al.* (2004) have shown that mouse NTS POMC neurons are activated by peripheral administration of CCK and by feeding-induced satiety, and that activation of the neuronal melanocortin-4 receptor (MC4-R) is required for CCK-induced suppression of feeding. In neuroanatomical support of this behavioural function, MC4-Rs have been localized to medial and commissural NTS subnuclei that are activated by gastrointestinal meal-related stimuli, such as distension and duodenal nutrient infusion (Mountjoy *et al.* 1994; Kishi *et al.* 2003).

The intracellular mediators of NTS neuronal integration of melanocortinergic and gut feedback signals have only begun to be examined. Recent work of Berthoud and colleagues (Sutton *et al.* 2004, 2005) has shown that extracellular signal-regulated kinase (ERK) 1/2 and cAMP response-element binding protein (CREB) increased upon peripheral administration of CCK and, pharmacological blockade of ERK phosphorylation blocks peripheral CCK-induced feeding inhibition.

This intracellular signalling cascade is also involved in the integration of melanocortin-receptor-mediated inputs to the caudal brainstem. The MC4-R agonist MTII rapidly and dose-dependently increased phosphorylation of both ERK 1/2 and CREB, and combination of brainstem fourth ventricular MTII and peripheral CCK increased the degree of ERK 1/2 phosphorylation compared to that produced by either stimulus alone.

Brainstem application of the MC4-R antagonist SHU9119 blocked CCK-induced increases in phospho extracellular signal-related kinase (pERK) and phospho-cAMP response element-binding protein (pCREB) and, consistent with a role for these MC4-R-induced ERK phosphorylation events in ingestion, increased food intake by increasing meal size. Pharmacological blockade of MAP kinase reduced MTII-induced ERK phosphorylation in the NTS and blocked the ability of MTII to suppress food intake. Furthermore, pre-treatment with the cAMP inhibitor, cAMP receptor protein-Rp isomer, significantly attenuated stimulation of pERK induced by either CCK or MTII.

Taken together, these findings demonstrate that activation of the ERK pathway is necessary for peripheral CCK and central MTII to suppress food intake. Thus, intracellular phosphorylation events, particularly those mediated by the cAMP→ERK→CREB cascade, represent novel targets for the investigation of the molecular basis of NTS integration of gut negative-feedback signals and centrally acting neuropeptides in the control of meal size.

5. SUMMARY/FUTURE DIRECTIONS

Both leptin and melanocortins act in the CNS to modulate the feeding inhibitory potency of meal-related gut negative-feedback signals. This modulation is paralleled by increased activation across and within individual NTS neurons. However, the full extent of these integrative properties as they apply to individual food-elicited signals in the gut remains to be characterized. The present data support the developing idea that integration is an essential feature of NTS neuronal processing. The widespread projection of visceral afferent information from the NTS to more rostral brainstem and forebrain sites along the gut–brain axis should be investigated to determine the extent and types of neural integration and its behavioural consequences of the control of food intake. It will also be important to pursue the identification of intracellular mediators underlying the integrative capacity of individual neurons both within the NTS and along this axis.

Alternative central feeding modulatory neuropeptide systems will also require investigation for their influence on brainstem integration of meal-related gut negative-feedback signals. For example, neuropeptide Y (NPY) has been shown to decrease the neurophysiological (but not behavioural) potency of gastric distension in

driving single NTS neurons (Schwartz & Moran 2002). NPY also blocks CCK-induced satiety and NTS c-fos activity, suggesting that NPY's actions on gut-sensitive NTS neurons may attenuate the feeding inhibitory effects of CCK (McMinn *et al*. 2000). It will be important to demonstrate the extent to which this NPY modulation extends to the full range of gut neural negative-feedback signals. Finally, Y1 receptors have been localized throughout the NTS sites that receive gut input, and on neurons that express MC4-Rs in the paraventricular hypothalamus and central nucleus of the amygdala (Kishi *et al*. 2005). These data support the potential comodulatory role of melanocortin and NPY in the NTS responses to meal-related gut stimuli and in the control of food intake.

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