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FEEDING-DEPENDENT DEPRESSION OF MELANIN-CONCENTRATING HORMONE AND MELANIN-CONCENTRATING HORMONE RECEPTOR-1 EXPRESSION IN VAGAL AFFERENT NEURONES

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Abstract—Food intake is regulated by signals from the gastrointestinal tract. Both stimulation and inhibition of food intake may be mediated by upper gastrointestinal tract hormones, e.g. ghrelin and cholecystokinin that act at least partly via vagal afferent neurones. We now report that vagal afferent neurones in both rat and man express melanin-concentrating hormone and its receptor, melanin-concentrating hormone-1R. In nodose ganglia from rats fasted for 24 h, RT-PCR revealed the expression of both melanin-concentrating hormone and melanin-concentrating hormone-1R, whereas in ganglia from animals fed *ad libitum* expression was virtually undetectable. Immunohistochemical studies also revealed expression of melanin-concentrating hormone and melanin-concentrating hormone-1R in nodose ganglion neurones of fasted rats, but signals were weak in rats fed *ad libitum*. Melanin-concentrating hormone and melanin-concentrating hormone-1R were expressed in the same neurones, a high proportion of which also expressed the cholecystokinin-1 receptor. When fasted rats were refed, there was down-regulation of melanin-concentrating hormone and melanin-concentrating hormone-1R expression over a period of 5 h. Similar effects were produced by administration of cholecystokinin to fasted rats. The cholecystokinin-1 receptor antagonist lorglumide inhibited food-induced down-regulation of melanin-concentrating hormone and melanin-concentrating hormone-1R. We conclude that the satiety hormone cholecystokinin acts on vagal afferent neurones to inhibit expression of melanin-concentrating hormone and its receptor. Since the melanin-concentrating hormone system is associated with stimulation of food intake this effect of cholecystokinin may contribute to its action as a satiety hormone. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: nodose ganglion, vagus, CCK.

Food intake is regulated by signals originating in both CNS and periphery (Schwartz et al., 2000). The latter include signals from adipocytes such as leptin, from the endocrine pancreas e.g. insulin, and from the gastrointestinal tract e.g. gut hormones (Druce et al., 2004; Dockray, 2004). Circulating molecules regulating appetite may act either directly on central neurones in regions of the brain where the blood brain barrier is leaky or absent, or may act indirectly via modulation of the discharge of afferent neurones. There is a substantial body of evidence to indicate that vagal afferent neurones in particular play a key role in the inhibition of food intake (Moran and Kinzig, 2004). These neurones can be excited by non-nutritive stimuli, e.g. gastric distension, and by hormones such as cholecystokinin (CCK), and potentiating interactions between the two have been reported (Schwartz et al., 1993).

CCK is produced in intestinal endocrine cells and acts at either CCK-1 (also called CCK-A) or CCK-2 (also called CCK-B, or gastrin/CCKB receptors) (Jensen, 2002). Both receptor types are expressed by vagal afferent neurones (Moriarty et al., 1997), but there is considerable evidence to indicate that it is the CCK-1 receptor population which is functionally important for inhibition of food intake (Reidelberger et al., 2003). Recent work has identified several other putative humoral agents that might act via vagal neurones that express the CCK-1 receptor. Thus the leptin receptor Ob-R is expressed by these neurones and there is potentiation between CCK and leptin for stimulation of afferent fiber discharge (Wang et al., 1997; Barrachina et al., 1997; Burdyga et al., 2002). In contrast, orexin type 1 (OX-R1) receptors expressed by these neurones are associated with inhibition of responses to CCK (Burdyga et al., 2003). Recently it was reported that cannabinoid CB1 receptors are also expressed by the same neurones (Burdyga et al., 2004). Interestingly CB1 receptor expression was depressed in rats fed *ad libitum* but was increased by fasting and down-regulated by refeeding in a CCK-dependent manner.

The receptors expressed by vagal afferent neurones implicated in control of food intake are also expressed by hypothalamic neurones. In addition there is a variety of other peptidergic transmitter systems found in hypothalamic neurones that have not so far been implicated in the peripheral signaling pathways that modulate food intake (Schwartz et al., 2000; Woods et al., 1998). One example is melanin-concentrating hormone (MCH) which is associated with stimulation of food intake (Forray, 2003; Collins and Kym, 2003). The cyclic nonadecapeptide MCH is increased in lateral hypothalamic neurones of fasted rats, and acts at the MCH-1R (Qu et al., 1996; Saito et al., 1999; Chambers et al., 1999). In the present study we asked whether MCH and MCH-1R are expressed in vagal afferent neurones. We report here evidence that there is increased expression of both MCH and MCH-1R in the

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Abbreviations: CCK, cholecystokinin; MCH, melanin-concentrating hormone; OX-R1, orexin type 1; PBS, phosphate-buffer saline; PFA, paraformaldehyde.

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nodose ganglion of fasted rats and that feeding decreases expression via CCK.

EXPERIMENTAL PROCEDURES

Tissues

Adult male Wistar rats (250–350 g) were maintained on standard rat chow and a 12-h light/dark cycle. Rats were killed by CO_2 inhalation and nodose ganglia rapidly dissected for studies described below. Experiments were approved by the relevant institutional review committee and conformed with national and international guidelines; the number of animals used was the minimum required to yield clear-cut conclusions. A human nodose ganglion was obtained from tissue taken during radical dissection of the neck for removal of a glomus tumor and processed as described below; the nodose ganglion was free of tumor although infiltration of the vagal nerve trunk could not be excluded (Burdyga et al., 2003). The latter work was approved by the Multi-Centre and Local Ethics Committee of Salford and Trafford Health Authority, and written consent was obtained.

Fasting–refeeding

In some experiments, rats were fasted for 24–48 h with water *ad libitum*. Some fasted rats were then refed for up to 5 h; others received CCK8 (Bachem, St. Helens, Merseyside, UK) by i.p. injection (10 nmol), or saline, and were killed up to 5 h later. In addition, some fasted rats received the CCK-1 receptor antagonist lorglumide (10 mg/kg) 15 min before refeeding and were killed 30 min or 5 h after refeeding. Nodose ganglia were dissected and processed as described below; in a few experiments gastric corpus was taken for comparison.

RT-PCR

Rat nodose ganglia pooled from five to six animals were extracted in Tri-Reagent (Sigma, Dorset, UK) as described previously (Burdyga et al., 2002). Briefly, RNA samples were treated with DNAase, reverse transcribed, and processed for PCR using BIOTAQ DNA polymerase (Bioline, London, UK) and the following primers:

1. Rat MCH-1R: sense, 5′-AACCCGGACACTGACCTCTACTGG-3′; antisense, 5′-TCGGGCGGCTGATGGACA-3′ (GenBank Acc. no. NM031758), 259 bp product.
2. Rat pro-MCH: sense, 5′-CGGCCTCCAAGTCCATCAG-3′; antisense, 5′-CTTCATCCCCAATTTCCCTCTT-3′ (GenBank Acc. no. M29712), 333 bp product.
3. GAPDH: sense 5′-GACCCCTTCATTGACCTCAACT-3′, antisense, 5′-CTCAGTGTAGCCCAGGATGCC-3′ (GenBank Acc. no. XO2231), 732 bp product.

The integrity of cDNA was confirmed by amplification of samples using primers to GAPDH. PCR products were gel purified (MinElute gel extraction kit, Qiagen, Crawley, UK) and sequenced

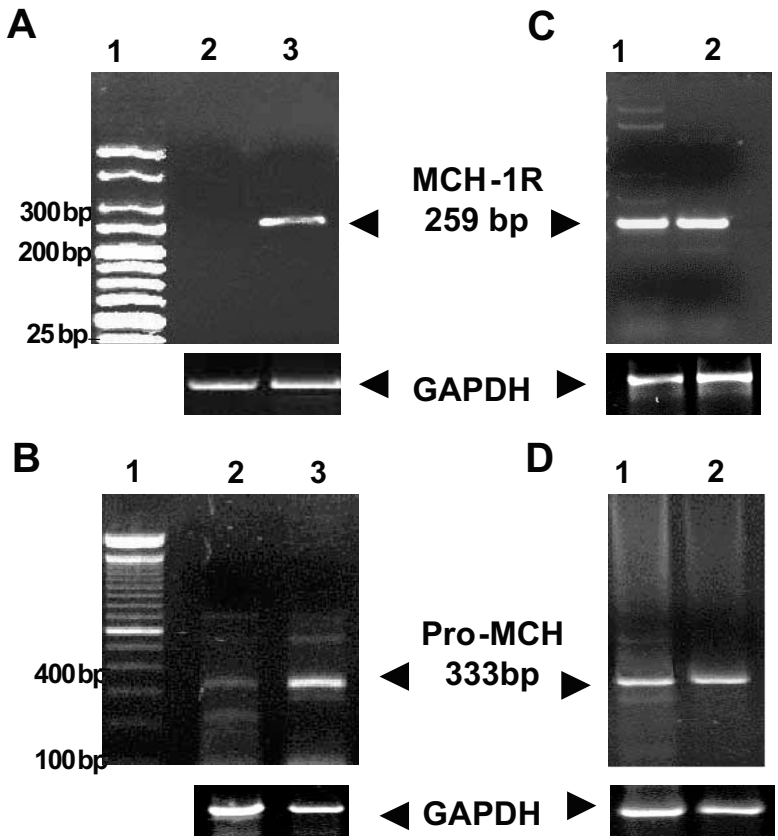

Fig. 1. RT-PCR indicates increased expression of MCH-1R and MCH in the nodose ganglia of fasted rats. (A) RT-PCR product of predicted size (259 bp) for rat MCH-1R in nodose ganglia from rats fasted for 24 h (lane 3) but not fed rats (lane 2) (pool of 12 ganglia in each case). (B) Similar data for MCH showing a band of the predicted size (333 bp) in the nodose ganglion of fasted (lane 3) but lower abundance in fed (lane 2) rats. (C, D) In gastric corpus there is also expression of MCH-1R and pro-MCH, respectively, but no difference in the abundance of RT-PCR products for material taken from fasted (lane 2) compared with fed rats (lane 1). Note similar abundances of GAPDH products. In panels A and (B) the lane 1 shows calibration markers.