Kevin Murphy
is a lecturer in the Department of Metabolic Medicine at Imperial College, University of London, UK.

Dissecting the role of cocaine- and amphetamine-regulated transcript (CART) in the control of appetite

K. G. Murphy

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Abstract

Cocaine- and amphetamine-regulated transcript (CART) codes for a neuropeptide system with a number of biological roles. The high conservation of CART across species suggests that it has an important role in mammalian physiology. CART is widely expressed in the central nervous system and the periphery, but is particularly concentrated in the hypothalamus. CART peptides, particularly CART (55–102), appear to have an important function in the regulation of energy homeostasis. This review aims to dissect the role of CART in appetite and energy expenditure. CART interacts with a number of central appetite circuits.

Hypothalamic CART expression is regulated by a number of peripheral factors, including the adipose hormone leptin. Intracerebroventricular administration of CART (55–102) reduces appetite and stimulates energy expenditure. Hypothalamic CART may also play an orexigenic role under specific circumstances, however, as injection of CART (55–102) into specific hypothalamic nuclei increases food intake.

Keywords: *cocaine-and amphetamine-regulated transcript, CART, hypothalamus, energy homeostasis, food intake, energy expenditure, neuropeptide, leptin*

INTRODUCTION

Recent years have brought tremendous advances in our understanding of the regulation of appetite and energy expenditure. The hypothalamus has long been considered to be the major central nervous system (CNS) region controlling energy homeostasis and, consequently, body weight. The hypothalamus receives neural and humoral signals which allow it to coordinate feeding behaviour and energy expenditure in response to conditions of altered energy balance. Extra hypothalamic regions, particularly the nucleus of the solitary tract (NTS) and the area postrema in the brain stem, also influence energy homeostasis.[1] The 'dual-centre' hypothesis of appetite regulation was first proposed by Hetherington and Ranson[2] in 1940 and then revisited by Anand and Brobeck in 1951.[3,4] Their hypothalamic lesioning experiments cast the ventromedial nucleus (VMN) of the hypothalamus as the appetite 'satiety' centre and the lateral hypothalamus (LH) as the 'feeding' centre. The integration of signalling between these regions was thought to regulate food intake, and disruption of this signalling to cause hyper- or hypophagia.

The concept of specific 'centres' controlling energy balance is now outdated. The modern model of the hypothalamic regulation of energy homeostasis postulates discrete neuronal pathways integrated into a complex neuronal network. This network roves through various hypothalamic nuclei and comprises numerous neurotransmitters and neuromodulators. The contentious role of cocaine- and amphetamine-regulated transcript (CART) peptides in appetite regulation is emblematic of the complexity of the energy homeostasis regulatory system, and highlights the difficulties implicit in teasing out the precise role of hypothalamic neuropeptides. This review attempts to

Kevin Murphy
Department of Metabolic Medicine,
Imperial College Faculty of Medicine,
Hammersmith Campus,
Du Cane Road,
London W12 ONN, UK

Tel: +44 (0)20 8383 2820
Fax: +44 (0)20 8383 3142
E-mail: k.g.murphy@imperial.ac.uk

dissect the role of CART in food intake and energy expenditure.

CART STRUCTURE AND DISTRIBUTION

In 1981, Spiess *et al.* isolated and partially sequenced what they called a somatostatin-like peptide from ovine hypothalamus.[5] Fourteen years later, using polymerase chain reaction differential displays, CART cDNA was isolated from rat brain as a transcript whose expression was regulated by acute administration of cocaine or amphetamine. The putative peptide coded for by the transcript contained the somatostatin-like peptide sequence, demonstrating that CART was translated into a peptide product.[6]

CART is highly conserved in mammals

CART mRNA and peptide are highly conserved between rodents and humans.[6,7] Immunohistochemical and *in situ* hybridisation studies in rats, primates and humans have revealed the presence of CART and CART peptides in a number of peripheral tissues and CNS regions. CART mRNA has been proposed as the third most abundant region-specific mRNA in the rat hypothalamus.[8] CART peptide immunoreactivity (CART-IR) is present in the same appetite-regulatory nuclei in the rat and in man: the LH, VMN, paraventricular nucleus (PVN), supraoptic nucleus (SON), dorsomedial nucleus (DMN) and the arcuate nucleus (ARC) (which is equivalent to the infundibular nucleus in humans).[9–11] It seems likely, therefore, that CART has similar functions in the human and rodent hypothalamus.

CART is widely distributed in the CNS, but is particularly concentrated in the hypothalamus

CART has been detected in the midbrain, frontal cortex,[7] amygdala, nucleus accumbens,[10] spinal cord, olfactory bulbs, adrenal medulla, retina, pituitary[9,12] and the pancreas.[13] CART-IR has also been detected in these areas,[14–16] as well as the NTS[17] and the myenteric plexus neurones of the ileum.[18] It is also found in general circulation at levels of approximately 50–150 pM,[13,19] and at ten-fold higher concentrations in hypothalamic–pituitary portal blood.[20]

In the rat, alternate splicing and polyadenylation of CART gives rise to four transcripts, the alternately spliced versions of which result in two different propeptides of 116 or 129 amino acids. The cleavage of a 27 amino acid signal sequence at the amino terminus of the CART propeptide results in peptides containing 89 and 102 amino acids, respectively. In human CART, no alternate splicing occurs and the mRNA is 92 per cent homologous with the shorter rat form, with 95 per cent homology at the amino acid level.[7] Following previously established nomenclature,[21] the CART peptides referred to in this review are numbered as if they are products of the longer 102 residue peptide unless otherwise stated. Note that, using this nomenclature, the putative active CART peptide fragment is designated CART (55–102), and that this peptide is identical to CART (42–89) described using the nomenclature based on the 89 residue peptide.

The CART amino acid sequence contains several pairs of basic residues, which are potential cleavage sites for prohormone convertase (PC) 1/3 and 2. PC2 appears to be important in generating CART (55–102) and CART (62–102), and PC1/3 in generating the CART fragments (33–102) and (10–89) from the long and short CART propeptides, respectively.[22] Cleavage at Lys-53-Arg-54 produces CART (55–102), which contains three disulphide bridges that are required for biological activity (see Figure 1).[21] It was initially isolated from ovine brain extract as described above.[5] Subsequently, CART (55–102) has been isolated from rat hypothalamus, nucleus accumbens and anterior pituitary.[23,24] The majority of work investigating the effects of CART on energy homeostasis has been performed using CART (55–102), although other fragments have also been investigated. Endogenous CART is processed tissue specifically and has been shown also to exist in the forms (1–102),

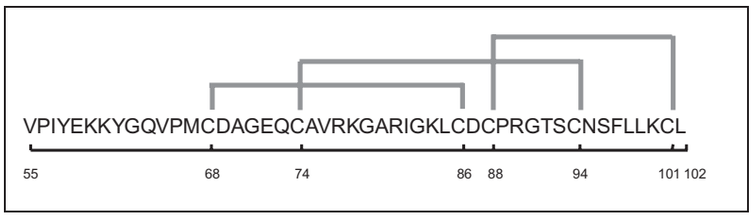

Figure 1: Diagram illustrating the positions of the disulphide bonds in human cocaine- and amphetamine-regulated transcript (CART) (55–102). Residue numbers are shown beneath the amino acid sequence. Rat and mouse CART (55–102) have an isoleucine rather than a valine at position 55, but are otherwise identical.

(10–102) and (62–102) in different rat tissues (see Figure 2).[23,24] All peptides isolated were the short versions, with no 13 amino-acid insert variants found, suggesting that the longer forms may not be physiologically relevant.

CART is involved in a number of physiological systems

BIOLOGICAL ROLES

CART is widely distributed in the CNS and the periphery, and appears to have a number of disparate physiological roles. It is expressed in a number of neuronal populations within the CNS, and is co-localised with known neurotransmitters and neuropeptides.[25–27] CART-IR has been demonstrated in the dense core vesicles of dendrites.[15] CART release from hypothalamic explants is calcium dependent.[28] The CART system therefore appears to represent a novel neurotransmitter or neuromodulatory system, although the CART receptor has not yet been identified.

Unsurprisingly, given the circumstances of its discovery, the role of CART in addictive behaviours, and in reward and reinforcement, has been widely studied. Cocaine addiction is thought to involve changes in the mesolimbic dopamine system, and CART has been reported to have a variety of effects on dopamine in the CNS.[16,29–33] There is also evidence that CART expression in the brain is reciprocally regulated by dopamine.[34] A recent study found that CART expression was increased in the nucleus accumbens of cocaine users.[35] CNS CART administration also influences nociception,[36–40] increases blood pressure[41,42] and has profound behavioural effects.[43–45] Strong evidence links CART to the hypothalamo–pituitary–adrenal (HPA) axis.[19,46–48] Intravenous CART administration has been shown to stimulate pancreatic exocrine secretion.[49]

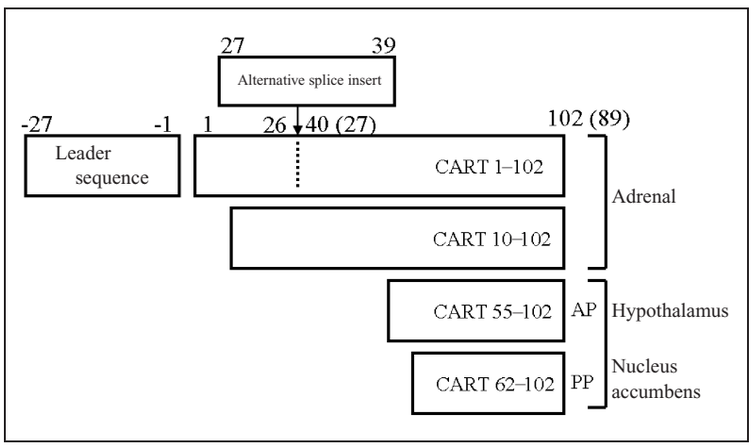

Figure 2: Diagram representing the tissue-specific processing of cocaine- and amphetamine-regulated transcript (CART) peptide. The numbers represent the amino acid residue at the termini of each peptide. Figures in brackets give the same information using nomenclature based on the short form of the peptide. AP, anterior pituitary, PP, posterior pituitary.

CART AND ENERGY HOMEOSTASIS

CART is expressed throughout the CNS, but is particularly concentrated in the hypothalamus, where it is found in nuclei important in the regulation of energy homeostasis.[11,14,25,28] CART mRNA has been proposed as the third most abundant region-specific mRNA in the rat hypothalamus.[8] In 1998, Kristensen *et al.* demonstrated that intracerebroventricular (ICV) injection of recombinant CART (55–102) inhibited feeding in normal and 24-hour fasted rats in a dose-dependent manner and suppressed the normal feeding response to neuropeptide Y (NPY).[21,50] Light-phase injection of 0.2 nmol CART (55–102) reduced feeding by approximately 50 per cent in non-fasted rats for the first hour post-injection, and 0.4 nmol reduced it almost to zero. ICV administration of CART antiserum

Intracerebroventricular administration of CART peptide reduces food intake

significantly increases night-time food intake, suggesting that CART peptide acts as an endogenous inhibitor of food intake.[21,51] The effects of ICV CART (55–102) on food intake have been confirmed by several groups,[13,47,50,52] and ICV injection of the smaller CART fragments (55–76) and (62–76) has also been shown to decrease food intake, although less potently than CART (55–102).[51] Chronic administration of 1 nmol/day CART (55–102) into the lateral ventricle reduced food intake and lowered the body weight of Sprague Dawley rats to 85 per cent of their initial weight. These effects lasted for three to five days before presumed tachyphylaxis developed and the animals began to eat the same amount of food as the vehicle-treated controls. The same treatment had a similar effect on the food intake and body weight of obese Zucker (*fa/fa*) rats, which have a leptin receptor defect.[53]

CART-knocked out mice

CART-deficient mice do not demonstrate the dramatically altered feeding behaviour or body weight that might be expected.[36,54] On a normal diet, CART-deficient mice have been reported to show a slight but consistent reduction in body weight and fat mass, although these trends did not reach significance.[36] On a high-fat diet, homozygous male and female CART-deficient mice show small but significant increases in weekly food consumption, body weight and fat mass compared with their wild-type littermates. Female heterozygous CART-deficient mice show similar differences, but male heterozygotes actually eat less than controls.[54] Differences in CART expression in the mesolimbic region have been described between wild-type male and female rats,[29] and gender-specific differences in the CART system may account for the different phenotypes of the male and female heterozygote CART-deficient mice. Developmental compensation may limit the consequences of CART deficiency. Despite considerable evidence that NPY plays a central role in stimulating appetite, NPY knockout mice eat and grow normally, suggesting that developmental compensation can occur in appetite regulatory systems.[55,56]

A heterozygous CART missense mutation, which replaces Leu-34 with a phenylalanine residue, co-segregates with severe obesity through three generations of a human family.[57] The ten-year-old boy in whom the mutation was first characterised had a normal birth weight but became obese at two years of age. His mother and a number of her relations also carried the mutation and had similar histories of early-onset obesity. The CART-deficient mouse also has a normal birth weight,[54] but it is difficult to compare the timing of the onset of obesity, and the diets, of the mouse and human models. As in the CART-deficient mouse model, the circulating leptin concentrations were appropriate to their fat mass; however, the differences between the male and female heterozygotes in the mouse model are not apparent in the human mutation, which seems to affect both sexes similarly. While there was no difference in the respiratory exchange ratios and energy expenditures of CART-deficient mice and wild-type littermates, both the boy and his mother had low resting metabolic rates. The effect of the human mutation on CART processing and release is not known, but it is interesting that it occurs in a part of the CART peptide that is not considered to be essential for the effects of CART on appetite. The effects of the human mutation may be more complicated than simple CART deficiency, perhaps influencing CART signalling only in specific tissues which produce longer forms of CART peptide. It is also possible that the differences between the mouse and human models reflect interspecies differences.

Other studies have tried and failed to find evidence of CART mutations in obese human populations.[58,59] There is evidence, however, that CART may influence fat distribution in humans[60] and that a polymorphism in the CART

promoter region is associated with obesity.[61]

CART AND CENTRAL APPETITE-REGULATORY CIRCUITS

CART is found in the hypothalamic arcuate nucleus, which is important in the regulation of energy homeostasis

The ARC is an important relay station for neural and circulating signals communicating with the hypothalamus. Situated at the base of the hypothalamus, the ARC is incompletely isolated by the blood–brain barrier, allowing circulating factors to access ARC neurones directly. Two major ARC neuronal populations regulate energy homeostasis: anorectic pro-opiomelanocortin (POMC) neurones, which co-express CART,[11,62] and orexigenic NPY and agouti-related peptide (AgRP) co-expressing neurones.[63] The POMC precursor molecule is cleaved to form the anorectic peptide, α-melanocyte-stimulating hormone (α-MSH), which inhibits feeding by agonising the melanocortin 3 (MC3) and melanocortin 4 (MC4) receptors.[64,65] Rodents or humans lacking the *POMC* gene, or with MC4 receptor mutations, are hyperphagic and obese.[66–70] AgRP is the endogenous antagonist of the MC3 and MC4 receptors. Central administration of AgRP increases food intake by blocking the endogenous anorectic tone of α-MSH at the MC3 and the MC4 receptors.[64] Central NPY administration also increases food intake.[71,72] These neurones project to other hypothalamic nuclei, including the PVN and the LH, and to extra-hypothalamic regions.

CART influences the release of other hypothalamic neuropeptides

The precise role of CART in the appetite regulatory network is still being elucidated. ICV injection of CART (55–102) activates neurones in the ARC and in other hypothalamic nuclei involved in the regulation of appetite, including the PVN, DMN and SON. CART co-localises with both anorectic and orexigenic neurotransmitters in the hypothalamus. The vast majority of ARC and retrochiasmatic area (RCA) POMC neurones also express CART, but CART co-localises with galanin in the PVN.[25] Almost all CART-IR in the LH and DMN is co-localised with melanin-concentrating hormone (although not with either of the orexins).[25,26] There are contradictory reports regarding the co-localisation of CART-IR and corticotrophin-releasing hormone (CRH).[25,73] CART-IR is also found in oxytocinergic and vasopressinergic perikarya in the magnocellular PVN and the SON.[25] CART mRNA and peptide co-localise with thyrotropin-releasing hormone (TRH) in the PVN;[26] and CART-IR and somatostatin co-localise in some periventricular neurones.[25] CART neurones in the PVN and the SON co-express dynorphin and a small number of CART neurones in the ARC co-express neurotensin.[11]

The co-expression of CART and POMC in the ARC is consonant with this neuronal population reducing food intake; however, CART suppresses the release of α-MSH from hypothalamic explants.[47] The anorectic actions of CART do not appear to depend on the melanocortin system, as blockage of the MC3 and MC4 receptors following central AgRP administration does not prevent CART-induced hypophagia.[74] Examining the effects of ICV CART on food intake in MC3 receptor- or MC4 receptor-deficient mice would prove the role of the melanocortin system in CART-induced anorexia more conclusively.

CART stimulates the release of AgRP from *ex-vivo* hypothalamic explants and AgRP significantly decreases the release of CART-IR in the same system.[75] AgRP activated ARC CART neurones[76] following ICV administration, however. CART has also been postulated to interact with the NPY system. CART-IR is not found in NPY neurones, but NPY-positive varicosities have been detected surrounding CART immunoreactive cell bodies in the PVN, DMN, LHA and ARC,[26,51] suggesting that NPY release may influence the firing of CART

neurones. NPY stimulates the release of CART-IR and CART (55–102) stimulates the release of NPY from hypothalamic explants.[75] The CART-induced release of orexigenic neuropeptides seems counterintuitive if CART is a simple anorectic agent, and this has been suggested to represent a negative feedback effect.[75] Although CART has been shown to prevent NPY-induced hyperphagia,[21,51] this does not necessarily suggest a direct connection between the systems. One might expect a number of anorectic neuropeptides to block NPY-induced feeding if administered at a sufficiently high dose.

The orexigenic neuropeptides prolactin-releasing peptide and galanin-like peptide decrease the release of CART from hypothalamic explants.[77,78] If CART sets an anorectic tone in the hypothalamus, this might account for the increase in appetite stimulated by these peptides. The suggested influence of CART on central dopamine and serotonin signalling may also partly explain the effects of CART on appetite.[32,79–82]

CART may act in the hindbrain to reduce food intake

The anorectic effects of CART, however, may be mediated through the hindbrain rather than the hypothalamus. Fourth ventricular injection of CART (55–102) reduces the short-term intake of sucrose solution, possibly due to conditioned taste aversion.[45,83,84] Plugging the cerebral aqueduct between the third and fourth ventricles blocks the anorectic effect of administration of 0.2 nmol CART (55–102) into the third ventricle.[45] ICV administration of CART peptides may therefore be activating — and ICV CART antibodies blocking — anorectic CART circuits in the hindbrain rather than the hypothalamus.[21,50] CART and CART immunoreactive neurones are found in brain stem nuclei involved in the control of gastrointestinal function, including the area postrema, the NTS and the parabrachial nucleus. Administration of 0.1 nmol CART (55–102) into the third ventricle, or 0.2 nmol into the fourth ventricle, activates brain stem neurones, notably in the NTS.[50,83,85] The vagus nerve transmits neural information from the upper gastrointestinal tract to the NTS. Vagal afferents containing CART-IR terminate in the NTS,[85] and vagal afferent neurones in the nodose ganglia express CART. CART-IR is also transported along the vagus nerve.[86] Vagal afferents carrying signals from the gastrointestinal tract may use CART as a neurotransmitter to mediate signals to the brain stem.[86] Injection of 0.2 nmol CART (55–102) directly into the NTS, however, has only a small, non-significant effect on food intake, suggesting that the hindbrain site of CART's action on food intake is not the NTS, and that CART release from vagal afferents does not play a major role in satiation.[85]

Injection of CART (55–102) into the cisterna magna inhibits food intake, gastric acid secretion and gastric emptying, possibly via CRH.[87] ICV CART (55–102) decreases colonic transit time via central CRH and peripheral cholinergic signalling.[88] It has been postulated that a delay in gastric emptying might explain the anorectic effects of CART;[87] however, experiments have shown that it is possible to separate the effects of CART (55–102) on gastric emptying and appetite.[89] CART may therefore centrally regulate gut motor function via CRH circuits that operate independently of those mediating the effects of CART on appetite. It is also possible that the CART-stimulated release of CRH has consequences for the HPA stress axis, and that the observed anorexia is a result of the activation of stress signalling pathways.

CENTRAL CART RESPONDS TO PERIPHERAL SIGNALS

Peripheral CART itself does not appear acutely to affect food intake. Intraperitoneal injection of relatively high doses of CART (55–102) does not affect food intake.[13,88] Circulating CART

peptide concentration has a diurnal pattern which positively correlates with circulating corticosterone in the rat, but the significance of this is currently unknown;[19] however, other circulating factors do influence central CART signalling.

The hypothalamic CART system is regulated by leptin

The adipose hormone leptin functions as the afferent arm of a body fat regulation loop, influencing feeding and energy expenditure via the hypothalamus. The anorectic actions of CART appear to operate downstream of leptin. Hypothalamic CART mRNA is regulated by leptin. Fasting, which is associated with a reduction in circulating leptin, significantly reduces CART mRNA expression in the ARC in the rat, and causes a smaller, non-significant reduction in the DMN.[21] Normal ARC CART mRNA levels are restored by leptin administration.[21,90] Some rodent models of obesity have altered CART expression. CART is almost absent from the ARC of *ob/ob* mice and *fa/fa* rats, and is restored to control levels by daily leptin administration in *ob/ob* mice.[21] Intravenous leptin induces Fos-IR in neurones containing CART mRNA in the RCA, ARC, DMN and the ventral premammillary nucleus.[11,62] Diet-induced obesity has been reported to reduce the number of CART peptide-containing neurones in the ARC[91] and rats with higher circulating leptin have higher ARC CART expression, independent of body fat.[92] In a separate experiment, however, mild hyperleptinaemia did not alter CART expression in the ARC.[93] A recent paper has also shown CART to mediate leptin-stimulated bone remodelling.[94] It seems likely, therefore, that the actions of leptin are mediated, at least partly, via the CART system.[21,62] It has been suggested that leptin may signal through CART to inhibit fat accumulation on a high-fat diet. Chronic ICV infusion of CART (55–102) increases lipid oxidation in obese rats,[95] and ICV CART (55–102) increases circulating non-esterified fatty acids and decreases lipoprotein lipase activity in adipose tissue.[92]

Post-prandial satiation appears to be partly mediated by various gut hormones,[96] while the gastric hormone ghrelin stimulates hunger.[97–99] There has been little study of the effects of gut hormones on central CART expression; however, a recent study showed that ICV administration of des-acyl ghrelin — a form of ghrelin previously believed to play no role in appetite — decreases food intake and gastric emptying. In addition, administering des-acyl ghrelin ICV every six hours for 12 hours significantly increased hypothalamic CART expression.[100] It has also been suggested that CART may act as an intermediary in the cholecystokinin post-prandial satiety pathway.[86]

Peripheral glucocorticoids influence body adiposity, fat distribution and the metabolic syndrome[101] and peripheral glucocorticoid concentrations influence hypothalamic CART levels. Adrenalectomy reduces CART expression in the PVN and ARC.[48,102,103] This reduction is blocked by glucocorticoid replacement.[48,103] Plasma CART levels correlate with circulating corticosterone;[19] however, these effects are perhaps more likely to be related to the role of CART in the HPA axis and may not reflect a physiological link between the HPA axis and appetite or obesity.

CART has also been implicated in the regulation of the reproductive axis[104–107] and, in accord with this, hypothalamic CART expression is influenced by testosterone.[108] Castration increases ARC and decreases PVN CART expression. These changes are reversed by testosterone administration.[108] It has been suggested that similar changes observed as rats age are due to decreases in circulating testosterone, and that testosterone administration can return gene expression to the levels seen in younger animals.[108,109] The effects are small, however, and may be due to other variables such as body weight.[91] It is

unclear whether the role of CART in the reproductive axis is relevant to its role in energy homeostasis.

Hypothalamic CART expression is also regulated by thyroid status. CART expression in the PVN is upregulated in hypothyroid and downregulated in hyperthyroid states,[110,111] suggesting that CART may have a physiological role in the promotion of energy expenditure.

CART AND ENERGY EXPENDITURE

CART influences energy expenditure by modulating sympathetic nervous outflow and the hypothalamo–pituitary–thyroid (HPT) axis. CART peptide is found in neurones that regulate the sympathetic nervous system and appears to modulate sympathetic function.[27,112–116] Hypothalamic CART/POMC neurones innervating sympathetic preganglionic neurones in the spinal cord are activated by leptin, which may contribute to leptin-stimulated energy expenditure and thermogenesis.[62] The expression of uncoupling protein-1 (UCP-1) allows brown adipose tissue (BAT) to dissipate energy as heat instead of ATP formation. Importantly, intra-PVN injection of CART upregulates the expression of UCP-1 in rat BAT, presumably increasing energy expenditure.[117]

CART and the thyroid axis

CART mRNA and peptide co-localise with TRH in the PVN, primarily in hypophysiotropic neurones.[26,118] CART-immunoreactive neurones extending from the ARC, the medulla and the brain stem densely innervate pro-TRH neurones in the parvocellular PVN.[118–120] ICV administration of CART (55–102) prevents the usual fasting-induced suppression of pro-TRH in the PVN, increases the TRH content of hypothalamic cell cultures and stimulates the release of TRH from hypothalamic explants.[47,118] Both plasma thyrotropin and free triiodothyronine (T_3) have been shown to rise 20 minutes after ICV injection of CART (55–102), but this change failed to reach statistical significance.[47] It is possible that an HPT feedback circuit regulates hypothalamic CART expression. Chronic T_3 or L-thyroxine treatment in rats decreased CART expression in the whole hypothalamus and PVN, respectively;[111,121] however, these changes may be secondary to reduced circulating leptin concentrations.

The effects of CART on energy expenditure are in accord with the actions of an anorectic neurotransmitter released when body weight or food intake is higher than required — that is, when there is a positive energy balance. The role of CART in appetite regulation may be more complex than initially thought, however, as recent studies have cast doubt on the model that treats CART as a purely appetite-inhibitory agent.

ARE THE ANORECTIC EFFECTS OF CART NON-SPECIFIC?

The anorectic effects of ICV CART may be secondary to its effects on behaviour and motor function. Injection of CART (55–102) at doses as low as 2 pmol into the third cerebral ventricle, and higher doses of CART (62–76) into the lateral cerebral ventricle, dose-dependently increase anxiety-like reactions.[43,122] Kristensen *et al.* noted that ICV administration of CART (55–102) at 0.2 and 0.4 nmol caused movement-associated tremor, but reported no changes in spontaneous locomotor activity levels when animals were monitored in isolated activity test chambers.[21] They noted, however, that ICV administration of 2.4 nmol/day (0.1 nmol/hour) CART (55–102) caused severe motor disturbances for the first four days of the experiment. After this, tachyphylaxis was presumed to reduce the effects on behaviour, food intake and body weight.[53] Animals in a parallel experiment receiving only 1 nmol/day CART (55–102) still showed a mild gait ataxia when forced to run.[53] ICV injection of CART (55–102) at doses of 0.2 nmol and 0.4 nmol is associated with marked abnormalities in behaviour,

causing animals to adopt a flattened body posture and exhibit movement-associated tremor;[44] it does not stimulate the increase in grooming or sleeping expected following administration of a physiological satiety factor.[123] The effects on meal patterns and licking microstructure also suggest that ICV CART (55–102) (0.2 and 0.4 nmol) may compromise overall motoric competence.[124] The presence of motor abnormalities and tremor in CART-treated animals suggest that the reduced food intake following ICV administration at these doses may be a consequence of these behavioural changes, rather than a true anorectic effect. These behavioural changes may be caused by the activation of CART circuits in the hindbrain rather than the hypothalamus.[45] It is not known if ICV administration of lower doses of CART (55–102) that cause small but significant reductions in food intake also cause behavioural side-effects.[50] In contrast to ICV administration, intra-ARC administration of CART (55–102) at doses up to 0.4 nmol is not associated with flattened body posture or movement-associated tremor.[44]

CART HAS AN OREXIGENIC ROLE

In direct contrast with ICV administration, Abbott *et al.* found that injection of 0.2 nmol CART (55–102) directly into the SON, anterior hypothalamic area, PVN, VMN, DMN, ARC or LH of 24-hour fasted rats significantly increased food intake.[44] This orexigenic response was more marked following injection into the VMN, DMN, ARC and LH, and was not apparent until one or two hours following injection. Injection of CART (55–102) into the VMN and ARC at a fifth of this dose (0.04 nmol) still increased food intake. Wang *et al.* had previously found that intra-PVN CART (55–102) (0.1 nmol) did not increase food intake, although they did find that it blocked the orexigenic effects of NPY.[125] Abbott *et al.* did not observe an orexigenic effect following injection of 0.04 nmol CART (55–102) into the PVN; however, it may be that the dose used by Wang *et al.* was too low to elicit orexigenic effects.[44,125]

Administration of CART (55–102) into specific hypothalamic nuclei increases food intake

Hypothalamic CART expression is reduced in rodent models lacking leptin or with reduced leptin activity,[21] suggesting that CART operates downstream of leptin signalling. The effects of CART on energy expenditure also seem to be antithetical to those expected of an orexigenic neurotransmitter.[125] These metabolic consequences appear less contradictory if CART is considered to play a role in cold adaptation. If CART is part of the body's system to increase heat production under temperature stress, it is quite logical that it should increase both food intake and heat production.

In accord with this, chronic intra-ARC administration of CART (55–102) to rats increases food intake, BAT UCP-1 expression and their thermogenic response to a β_3-agonist. Overexpression of ARC CART using stereotactically targeted gene transfer also increased food intake. These CART overexpressing animals lost more weight than controls following a 24-hour fast, suggesting higher energy expenditure, and also had higher UCP-1 expression levels in BAT. Chronic cold exposure dramatically increases ARC CART expression which, coupled with the orexigenic and thermogenic effects of CART, suggests that ARC CART is important in cold adaptation.[126] While the pertinence of these results to adult humans (who have little BAT) may be limited, administration of CART (55–102) into the PVN also upregulates expression of the UCP-1 homologues UCP-2 and UCP-3 in white adipose tissue and muscle, respectively.[117] The roles of UCP-2 and UCP-3 in thermogenesis is controversial, but current evidence suggests that they have a role in fat metabolism and that they may therefore be more relevant to energy homeostasis in humans than UCP-1.[127]

The delayed feeding response to intranuclear CART (55–102)[44] contrasts

with the immediate action of the majority of orexigenic neuropeptides.[128–131] The mechanism for this delayed orexigenic action is not clear. CART (55–102) may influence the release of other appetite effectors within the hypothalamus, possibly stimulating the release of orexigenic peptides and/or reducing the release of anorexigenic peptides to cause its eventual effect on appetite. As mentioned above, CART (55–102) has been demonstrated to stimulate the release of NPY and AgRP, and to reduce the release of α-MSH, from basal hypothalamic explants.[47,75] An orexigenic role for CART would be in accord with the increased CART-IR seen in discrete hypothalamic regions in fasted rats[132] and with the slightly lower body weight and fat mass of CART-deficient mice mentioned above.[36,54] As CART is thought to influence the stress response, it is also possible that this lowered body weight is a consequence of a disrupted HPA axis.

Distinct CART circuits may increase and decrease appetite

CART may have different functions in the hypothalamus and the hindbrain.[45,133] There may also be distinct CART appetite circuits within the hypothalamus, signalling in both orexigenic and anorectic capacities. The PVN is innervated by CART neurones from a number of CNS regions, including the ARC, the RCA, the LH, the parabrachial nucleus and the NTS,[119] and may therefore mediate a number of different functions and signals. CART (55–102) has been shown to have inimical effects on the inhibitory gamma-aminobutyric acid-ergic synaptic inputs into two putatively different populations of adipostat neurones in the medial parvocellular PVN.[134] Alternatively, injection of CART (55–102) into discrete hypothalamic nuclei may activate an inhibitory autoreceptor that downregulates anorectic CART signalling.

CONCLUDING REMARKS

The CART system appears to assume multiple roles in energy homeostasis, depending on the internal and external milieu. Certainly, it seems viable that CART, as an intermediary in what appear to be several separate neuronal systems,[135] might well transmit opposing messages between different neuronal populations in diverse regions of the hypothalamus. Further electrophysiological and *in vivo* experiments may help to explain just how, and under which conditions, CART controls appetite and energy expenditure. The development of specific CART antagonists would be very useful in dissecting these multiple roles. Unfolded, recombinant CART peptide (55–102) increases food intake when administered into the lateral ventricle, an effect consistent with that of a CART antagonist.[136] Until the CART receptor is identified or a reliable binding study established, however, it cannot be assumed that the unfolded peptide represents a specific CART receptor antagonist.

Ironically, recent studies have suggested that CART expression is not influenced by psychoactive drugs.[30,137,138] Three years after its initial discovery, CART (55–102) was reported as a hypothalamic satiety factor,[21,51] and it is this finding that has provided a focus for much subsequent CART research. The misleading acronym, however, endures. Perhaps completion of the definitive experiments required to dissect the role of the CART system in energy homoeostasis will prompt a reassessment of the name of this conserved appetite regulatory transcript.

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