

Overexpression of CART in the PVN Increases Food Intake and Weight Gain in Rats

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Objective: Cocaine- and amphetamine-regulated transcript (CART) codes for a hypothalamic neuropeptide, CART (55–102), which inhibits food intake. Intracerebroventricular injection of CART (55–102) reduces appetite, but also results in motor abnormalities. More recently, studies have demonstrated that administration of CART directly into the paraventricular nucleus (PVN) increases food intake. To investigate the role of CART in the regulation of energy balance in the PVN, we used recombinant adeno-associated virus (rAAV) to overexpress CART in the PVN.

Methods and Procedures: Male Wistar rats were injected with either rAAV-encoding CART (rAAV-CART) or rAAV-encoding enhanced green fluorescent protein (rAAV-EGFP) as a control. Food intake and body weight were measured regularly. Animals were fed on normal-chow diet for the first 93 days of the study. After this point, they were fed on high-fat diet. Animals were killed 138 days postinjection and blood and tissues were collected for analysis.

Results: Overexpression of CART in the PVN resulted in increased cumulative food intake and body weight gain compared with rAAV-EGFP controls when fed normal chow. These changes became significant at day 65 and 79, respectively and were accentuated on a high-fat diet. A 4% increase in food intake was observed in rAAV-CART animals on a normal-chow diet and a 6% increase when fed a high-fat diet. At the end of the study, rAAV-CART-treated animals had higher circulating leptin concentrations in accord with their higher body weight.

Discussion: These data provide further evidence that hypothalamic CART plays an orexigenic role.

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INTRODUCTION

Cocaine- and amphetamine-regulated transcript (CART) was initially discovered as a transcript upregulated in the rat brain in response to the administration of cocaine or amphetamine (1). CART is abundant in the hypothalamus (2,3), where it is highly expressed in the paraventricular (PVN) and arcuate (ARC) nuclei (4). Intracerebroventricular (ICV) administration of CART peptides results in neuronal activation within the PVN and ARC (5). These nuclei are critical in the regulation of energy homeostasis (6). The ARC receives and integrates peripheral signals of nutritional status. The PVN processes information from the ARC and other hypothalamic nuclei to regulate both appetite and energy expenditure.

In 1998, Kristensen *et al.* reported that CART was an anorectic neuropeptide. ICV administration of the endogenously occurring CART fragment, CART (55–102), at doses of 0.2 or 0.4 nmol reduces food intake in normal rats (7). Several other groups subsequently demonstrated the anorectic effect of ICV CART (55–102) (refs. 5,8–10). In addition, Qing and Cheg have recently demonstrated that overexpression of CART after administration of recombinant adeno-associated

virus (rAAV)-CART into the third ventricle attenuates body weight gain in diet-induced-obese rats (11). However, studies in which CART is injected or overexpressed in discrete hypothalamic nuclei suggest that the CART system may also play an orexigenic role in the hypothalamic feeding circuits (12,13).

Kristensen *et al.* noted that ICV administration of CART (55–102) caused movement-associated tremor, but reported no changes in spontaneous locomotor activity levels when animals were monitored in isolated activity-test chambers (7). However, long-term ICV administration of 2.4 nmol/day CART (42–89) has been shown to cause severe motor disturbances and animals receiving only 1 nmol/day CART (42–89) still demonstrate a mild gait ataxia when forced to run (14). ICV injection of CART (55–102) at doses of 0.2 and 0.4 nmol is associated with marked abnormalities in behavior, causing animals to adopt a flattened body posture, and exhibit movement-associated tremor (12) and does not stimulate the increase in grooming or sleeping expected after administration of a physiological satiety factor (15). The effects of the same doses of ICV CART on meal pattern and licking microstructure suggest that overall motoric competence is compromised (16). The presence

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of motor abnormalities and tremor in CART-treated animals suggests that the anorectic effects of ICV CART (55–102) may be a consequence of these behavioral changes, rather than the activation of specific anorectic circuits. These behavioral changes may be caused by the activation of CART circuits in the hind-brain rather than the hypothalamus (17).

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 (12). Direct administration of CART (55–102) into discrete hypothalamic nuclei increases food intake suggesting that CART may play an orexigenic role in the regulation of energy homeostasis (12).

In accord with this finding, we subsequently demonstrated that chronically upregulating CART signaling in the ARC increases cumulative food intake and weight gain (13). To investigate further the role of CART in appetite regulation, we have examined the effect of upregulating CART signaling in the PVN. AAV is a small, nonpathogenic, single-stranded DNA virus, which has been used in animals and humans as a vector for gene transfer (18). Type 2 AAV has a tropism for neurons and rAAV has been used to introduce novel genes into specific areas of the central nervous system (19) with sustained gene expression (20). rAAV has been used previously to investigate the role of a number of neuropeptides or proteins in the hypothalamic regulation of energy homeostasis, including leptin (21), α -melanocyte-stimulating hormone (α -MSH) (22), and neuropeptide Y (NPY) (23). We describe here the use of rAAV to overexpress CART in the PVN of male Wistar rats and the observed effects on food intake and body weight.

METHODS AND PROCEDURES

Materials

The reagents for the hypothalamic explant experiments were purchased from BDH (Poole, UK). Cell culture materials were supplied by Life Sciences Technology (Paisley, UK) and all other reagents by Sigma Chemical (Poole, UK).

Viral preparation and titre

The rAAV-CART and rAAV-encoding enhanced green fluorescent protein (rAAV-EGFP) were prepared using the adenovirus free method (24) as previously described (23). HEK293 cells were transfected with the rAAV plasmid and pDG was provided by Dr Kleinschmidt, Germany (24). The rAAV plasmid used, pTRCGW, carried the full length rat CART (u10071) or EGFP transgene and the AAV-inverted terminal repeats (a gift from J Verhaagen at the Netherlands Brain Institute, Amsterdam, The Netherlands). The rAAV-CART and rAAV-EGFP particles produced after cell transfection were released by cell lysis (3 freeze/thaw cycles). Viral particles were purified by heparin sulfate chromatography followed by a continuous iodixanol gradient and the viral particle titre was quantified by dot-blot analysis (25).

Animals

Male Wistar rats 250–300 g, housed in single cages (specific pathogen free, Imperial College London, UK) were maintained under a controlled environment (temperature 21–23°C, 12-h light–dark cycle, lights on at 07:00) with *ad libitum* access to food (RM1 diet; SDS UK, Witham, Essex, UK) and water. All animal procedures

were approved by the British Home Office Animals (Scientific Procedures) Act 1986 (Project Licence no. 70/5195).

Intranuclear injection

Male Wistar rats were anesthetized with intraperitoneal xylazine (Bayer, Bury St Edmunds, UK) and ketamine (Parke-Davis, Gwent, UK) and held in a Kopf stereotaxic frame. Each animal received an injection of 1 μ l rAAV-CART or rAAV-EGFP, containing 10^6 viral particles, over 5 min, under aseptic conditions, via a burr hole using a 33-gauge stainless steel injector (Plastics One, Roanoke, VA) and an infusion pump (Harvard Apparatus, Edenbridge, UK). The PVN injection coordinates were calculated using the Paxinos and Watson rat brain atlas (26) as 1.8 mm posterior to bregma, 0.3 mm lateral to bregma, and 8 mm below the skull surface. Animals were allowed to recover for seven days post-surgery before food and body weight measurements commenced.

The effect of CART overexpression in the PVN

Male Wistar rats ($n = 50$) were injected into the PVN with either rAAV-CART or rAAV-EGFP. Twenty-eight days postinjection, 10 animals from each group were killed to ensure increased hypothalamic CART release (see study 1 below). The remaining animals were maintained for 138 days postinjection (see study 2 below).

Study 1—Effect of intra-PVN injection of rAAV-CART on hypothalamic CART and neuropeptide release. Male Wistar rats were injected into the PVN with either rAAV-CART ($n = 10$) or rAAV-EGFP ($n = 10$). Twenty-eight days postinjection, the release of CART-immunoreactivity (CART-IR) from the hypothalamus was measured using a static incubation system using methods previously described (27). The release of the hypothalamic neuropeptides, NPY, agouti-related peptide (AgRP), and α -MSH was also measured. Rats were killed by decapitation and the brain removed. A 1.7 mm slice, including the entire PVN, was taken from the basal hypothalamus using a vibrating microtome (Microfield Scientific, Dartmouth, UK) and transferred to artificial cerebrospinal fluid. The artificial cerebrospinal fluid was collected after a 1-h basal incubation and a subsequent 1-h exposure to artificial cerebrospinal fluid containing 56 mmol/l potassium and stored at -20°C until measurement of neuropeptide immunoreactivity by radioimmunoassay. Explants showing smaller neuropeptide release response to the hyperkalemic period than the basal period were excluded from analysis ($<10\%$).

Study 2a—Effect of intra-PVN injection of rAAV-CART on food intake and body weight. Male Wistar rats were injected, as described above, with rAAV-CART ($n = 15$) or rAAV-EGFP ($n = 12$) into the PVN. Rats were given *ad libitum* access to standard rat chow (RM1 diet SDS UK), which contains 7.5% kJ as fat. Food intake and body weight were measured three times a week. At 93 days postinjection, both experimental groups were placed onto a high-fat diet which contained 45% kJ as fat (Research Diets, New Brunswick, NJ). The study was ended 138 days postinjection.

Study 2b—Effect of intra-PVN injection of rAAV-CART on hypothalamic neuropeptide expression, plasma hormones, fat pad weight, and BAT UCP-1 expression. At 138 days postinjection of rAAV into the PVN, rats were killed by CO_2 asphyxiation. Hypothalami were block dissected, frozen in liquid nitrogen and stored at -70°C until RNA extraction. Gene transfer was confirmed by ribonuclease protection assay (RPA) for woodchuck post-transcriptional regulatory element (WPRE), part of the rAAV construct (28). RPA analysis confirmed gene transfer in 12–15 rats per group. In 2 animals, whole brains were taken, mounted and snap frozen, and stored at -70°C before localization of WPRE mRNA expression by *in situ* hybridization. Hypothalamic expression of AgRP, NPY, and pro-opiomelanocortin (POMC) was measured by RPA. Blood was collected by cardiac puncture into plastic tubes containing potassium EDTA (final concentration of 1.2–2 mg EDTA/ml blood) (Sarstedt, Leicester, UK) and

aprotinin 2000 KIU/tube (Bayer). Plasma was separated by centrifugation, immediately frozen and stored at -70°C until radioimmunoassay. Epididymal fat pads were dissected and weighed. Interscapular brown adipose tissue (BAT) was dissected free, weighed, snap frozen, and stored at -70°C until RNA extraction and measurement of uncoupling protein-1 (UCP-1) expression by RPA.

Radioimmunoassays

CART, NPY, AgRP, and α -MSH immunoreactivity were measured using established methods (29). Thyroid stimulating hormone and luteinizing hormone levels were assayed using reagents and methods provided by the National Institute of Diabetes and Digestive and Kidney Diseases and the National Hormone and Pituitary Program (Dr Parlow; Harbor University of California, LA Medical Center, CA) (30). Plasma leptin was measured using methods and reagents supplied by Linco Research. (St Charles, MO).

RPAs

RNA was extracted from individual frozen hypothalami or interscapular BAT using Tri-Reagent (Helena Biosciences, Sunderland, UK) according to the manufacturer's protocol, as previously described (31). Quantification of UCP-1, NPY, AgRP, and POMC mRNA was performed using Ambion RPA III kit (Ambion, TX). The UCP-1 riboprobe corresponded to nucleotides 351–658 (UCP-1 m11814). The NPY riboprobe corresponded to nucleotides 81–538 (NPY m15880). The AgRP riboprobe corresponded to nucleotides 16–361 (AgRP u89484). The POMC riboprobe corresponded to nucleotides 185–674 (POMC nm_139326). Rat β -actin (Ambion, Warrington, UK) was used as an internal control to correct for RNA loading. RNA was hybridized overnight at 42°C with 1.3×10^5 Bq ^{32}P [CTP]-labeled riboprobe. Reaction mixtures were digested with RNase A/T1, the protected fragments precipitated and separated on a 4% polyacrylamide gel. The dried gel was exposed to a phosphorimager screen overnight and bands quantified by image densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

In situ hybridization

In situ hybridization using antisense WPRE riboprobe (corresponding to nucleotides 1181–1489 of the woodchuck hepatitis genome (J04514)) was performed on $15\text{ }\mu\text{m}$ sections, as previously described (13). Slides were hybridized overnight at 60°C with 20×10^3 Bq ^{35}S CTP-labeled probe added to each slide. Subsequently, slides were RNase-A treated, washed, ethanol dehydrated. Sections were then dried and dipped in Hypercoat LM-1 Nuclear Emulsion (GE Healthcare, Buckinghamshire, UK). Sections were exposed for 10 days at 4°C before being developed. Slices were then fixed in acid alcohol and incubated in cresyl violet. They were then dehydrated through increasing concentrations of ethanol, cleared in xylene and cover-slipped.

Statistics

Values are all shown as mean \pm s.e.m. as well as median and range. Data from study 1 was analyzed using a Mann–Whitney test between treatment and control groups. Cumulative food intake and body weight data were analyzed using generalized estimating equations with exchangeable correlation matrix and robust standard errors (Stata 8; Statacorp LP, College Station, TX). End-point data from study 2 was analyzed using a Mann–Whitney test between treatment and control groups.

RESULTS

Study 1—Effect of intra-PVN injection of rAAV-CART on hypothalamic CART and neuropeptide release. CART release from hypothalamic explants was increased after intra-PVN rAAV-CART injection compared to controls (CART [fmol/explant mean \pm s.e.m. (median [interquartile range])] 65.8 ± 8.4 (2.9 [2.0:3.3]) rAAV-EGFP vs. 142.9 ± 33.7 (4.5 [3.5:5.8])

rAAV-CART. $P < 0.05$ $n = 9$) (Figure 1a). Tissue viability of the explants was confirmed by an increased release of CART during incubation in 56-mmol/l potassium compared to the basal release.

NPY release from hypothalamic explants was increased after intra-PVN rAAV-CART injection compared with rAAV-EGFP controls (NPY [fmol/explant] 19.3 ± 5.4 (0.8 [0.37:0.96]) rAAV-EGFP vs. 68.8 ± 13.4 (2.7 [1.9:4.3]) rAAV-CART, $P < 0.01$ $n = 7$) (Figure 1b). AgRP release from hypothalamic explants was also increased after intra-PVN rAAV-CART injection compared with rAAV-EGFP controls (AgRP [fmol/explant] 4.3 ± 1.0 (3.9 [2.4:6.6]) rAAV-EGFP vs. 10.1 ± 1.6 (10.2 [8.7:12.4]) rAAV-CART, $P < 0.05$ $n = 7$) (Figure 1c). α -MSH release from hypothalamic explants was not significantly altered after intra-PVN rAAV-CART injection compared to rAAV-EGFP controls (α -MSH [fmol/explant] 1.6 ± 0.4 rAAV-EGFP (1.1 [0.5:2.0]) vs. 1.2 ± 0.2 (1.2 [0.9:1.3]) rAAV-CART $n = 7$) (Figure 1d).

Study 2a—Effect of intra-PVN injection of rAAV-CART on food intake and body weight. There were no differences in body weight between the two groups at the start of the study (preinjection body weight [g] 309.0 ± 5.7 (309 [298.5:323.3]) rAAV-EGFP vs. 316.9 ± 5.1 (315 [305.5: 333.5]) rAAV-CART). Following injection, animals were fed on a normal-chow diet. By day 64 postinjection, rAAV-CART animals had gained significantly more weight than controls (cumulative body weight gain [g] 145.6 ± 3.7 (143.5 [138.5:151]) rAAV-EGFP vs. 160.5 ± 5.8 (164 [150:173]) rAAV-CART, $P < 0.05$ $n = 12$ –15) (Figure 2a). By day 79 postinjection, rAAV-CART animals had consumed significantly more chow than controls (cumulative food intake [MJ] 34.9 ± 0.6 (34.4 [33.6:36.3]) rAAV-EGFP vs. 36.1 ± 0.4 (35.9 [35.2:36.8]) rAAV-CART,

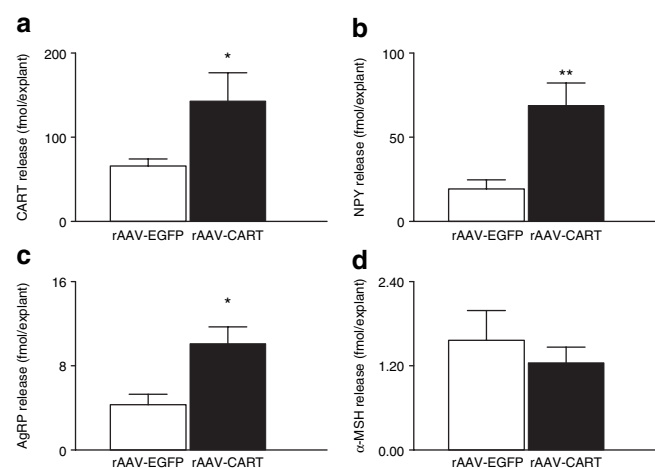


Figure 1 Release of (a) cocaine- and amphetamine-regulated transcript (CART), (b) neuropeptide Y (NPY), (c) agouti-related peptide (AgRP), and (d) α -melanocyte-stimulating hormone (α -MSH) from hypothalamic explants, 4 weeks after intra-paraventricular nucleus injection of $1\text{ }\mu\text{l}$ of recombinant adeno-associated virus-encoding enhanced green fluorescent protein (rAAV-EGFP) (open bars) or rAAV-CART (closed bars). Values are shown as fmol/explant mean \pm s.e.m., CART $n = 9$, NPY $n = 7$, AgRP $n = 8$, and α -MSH $n = 7$. * $P < 0.05$ and ** $P < 0.01$ vs. rAAV-EGFP.

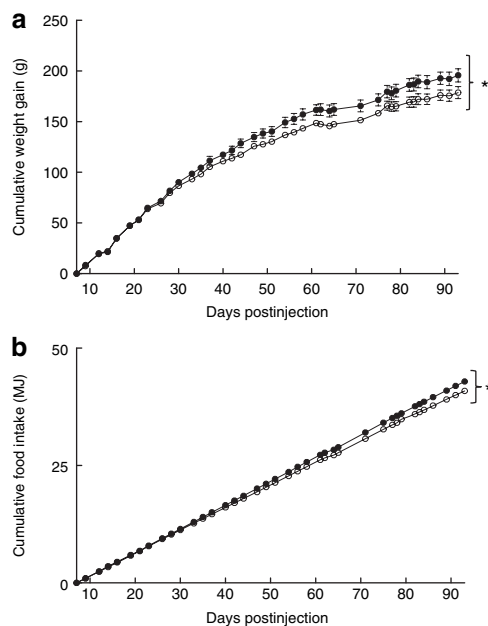


Figure 2 Cumulative (a) body weight gain and (b) food intake (day 7–92) after intra-paraventricular nucleus injection of 1 μ l recombinant adeno-associated virus-encoding enhanced green fluorescent protein (rAAV-EGFP) (open circles) or rAAV-encoding cocaine- and amphetamine-regulated transcript (rAAV-CART) (closed circles). Animals were fed on a normal-chow diet ($n = 12$ –15) after confirmation of gene transfer by woodchuck post-transcriptional regulatory element ribonuclease protection assay. Values are shown as mean \pm s.e.m. Trend analysis revealed that rAAV-CART animals ate significantly more and gained more weight than rAAV-EGFP controls over the 93-day period $*P < 0.05$ vs. rAAV-EGFP.

$P < 0.05$ $n = 12$ –15) (Figure 2b). The rAAV-CART animals showed a 4% increase in cumulative food intake compared to rAAV-EGFP controls up to 93 days postinjection (cumulative food intake [MJ] 40.9 ± 0.7 [40.9 [39.6:43.1]] rAAV-EGFP vs. 42.9 ± 0.5 [42.6 [41.9:43.7]] rAAV-CART, $P < 0.05$ $n = 12$ –15). Trend analysis revealed that over the 93-day period the rAAV-CART animals ate significantly more food and gained significantly more weight than rAAV-EGFP control animals ($P < 0.05$ $n = 12$ –15). On day 94, animals were transferred onto a high-fat diet. Over the duration of the high-fat feeding period the rAAV-CART animals gained significantly more weight and ate significantly more food than rAAV-EGFP controls. Between day 94 and day 138 of the study, rAAV-CART animals showed a 6% increase in cumulative food intake compared to rAAV-EGFP controls (cumulative food intake [MJ] 73.3 ± 1.3 [73.7 [70.4:77.6]] rAAV-EGFP vs. 78.0 ± 0.9 [77.4 [75.4:79.8]] rAAV-CART $P < 0.05$ $n = 12$ –15 (Figure 3b)), (cumulative body weight gain [g] 244.7 ± 9.0 [247 [230:256]] rAAV-EGFP vs. 281.1 ± 10.8 [276 [259:305]] rAAV-CART $P < 0.05$ $n = 12$ –15 (Figure 3a)). No behavioral or motor abnormalities were observed at any point during the study. *In situ* hybridization for WPRE was used to localize transgene expression in brains from two animals. Transgene expression was observed in the PVN (see Supplementary Figure S1 online).

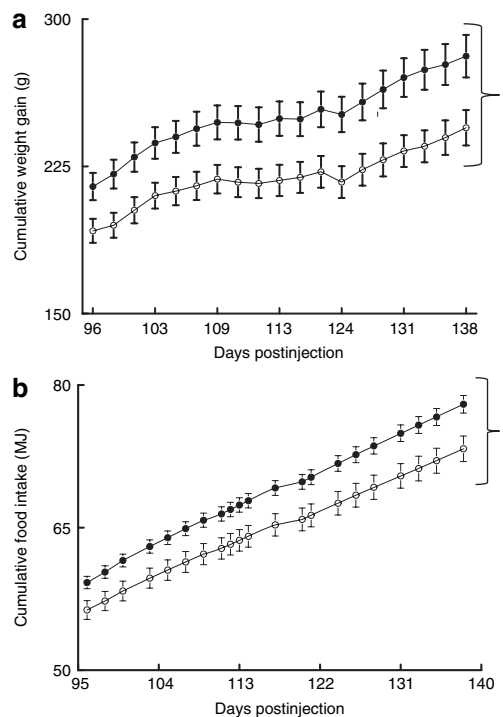


Figure 3 Cumulative (a) body weight gain and (b) food intake (day 93–138) after intra-paraventricular nucleus injection of 1 μ l recombinant adeno-associated virus-encoding enhanced green fluorescent protein (rAAV-EGFP) (open circles) or rAAV-encoding cocaine- and amphetamine-regulated transcript (rAAV-CART) (closed circles). Animals were fed on a high-fat diet ($n = 12$ –15) after confirmation of gene transfer by woodchuck post-transcriptional regulatory element ribonuclease protection assay. Values are shown as mean \pm s.e.m. Trend analysis revealed that rAAV-CART animals ate significantly more and gained more weight than rAAV-EGFP controls over the 45-day period $*P < 0.05$ vs. rAAV-EGFP.

Study 2b—Effect of intra-PVN injection of rAAV-CART on plasma hormones, hypothalamic neuropeptide expression, fat pad weight, and BAT UCP-1 expression. Plasma leptin concentrations were elevated in rAAV-CART-treated animals compared to rAAV-EGFP controls (leptin [ng/ml] 16.1 ± 1.4 [15.1 [13.1:17.9]] rAAV-EGFP vs. 22.0 ± 2.1 [21.5 [18.3:25.8]] rAAV-CART $P < 0.05$ $n = 12$ –15) (Figure 4). There was no difference in the weight of the epididymal fat pad in rAAV-CART animals compared to controls (white adipose tissue [mg white adipose tissue/g body weight] 26.4 ± 1.8 [25.1 [21.3:30.7]] rAAV-EGFP vs. 28.0 ± 1.3 [27.5 [24.3:31.2]] rAAV-CART $P = 0.5$ $n = 12$ –15). There was no significant difference in the interscapular BAT pad weight in the rAAV-CART-treated animals compared to rAAV-EGFP controls (BAT [mg BAT/g body weight] 1.3 ± 0.1 [1.2 [1.0:1.5]] rAAV-EGFP vs. 1.5 ± 0.1 [1.6 [1.3:1.9]] rAAV-CART $P = 0.09$ $n = 12$ –15). There was no difference in the BAT UCP-1 mRNA expression between the two groups (BAT UCP-1 mRNA relative optical density [arbitrary units] 2.60 ± 0.29 [2.5 [2.2:3.4]] rAAV-EGFP vs. 2.58 ± 0.18 [2.4 [2.1:3.0]] rAAV-CART). Hypothalamic expression of AgRP, NPY, or POMC was the same in the two groups (relative optical density [arbitrary units] rAAV-EGFP vs. rAAV-CART: AgRP 0.25 ± 0.03 [0.22

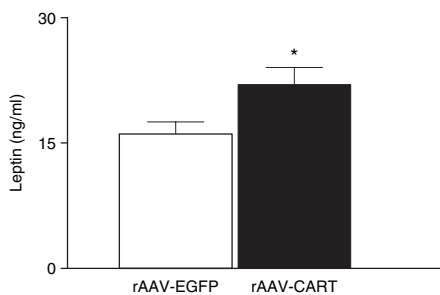


Figure 4 Plasma leptin concentration, 138 days after intra-paraventricular nucleus injection of 1 μ l adeno-associated virus-encoding enhanced green fluorescent protein (rAAV-EGFP) (open bars) or rAAV-encoding cocaine- and amphetamine-regulated transcript (rAAV-CART) (closed bars). Values are shown as ng/ml mean \pm s.e.m. $n = 12$ –15. * $P < 0.05$ vs. rAAV-EGFP.

[0.20:0.23]) vs. 0.21 ± 0.02 (0.22 [0.17:0.24]); NPY 0.41 ± 0.06 (0.33 [0.29:0.47]) vs. 0.32 ± 0.03 (0.31 [0.26:0.37]); POMC 0.61 ± 0.03 (0.60 [0.53:0.68]) vs. 0.63 ± 0.06 (0.56 [0.49:0.70]). Plasma thyroid stimulating hormone levels did not differ between the two groups (Plasma thyroid stimulating hormone [ng/ml] 2.2 ± 0.1 (2.3 [2.0:2.4]) rAAV-EGFP vs. 2.5 ± 0.2 (2.3 [2.0:2.8]) rAAV-CART).

DISCUSSION

We have used rAAV to overexpress CART in the PVN of the hypothalamus in an attempt to elucidate the role of CART in the regulation of food intake and body weight. Injection of rAAV-CART into the PVN of adult rats resulted in increased hypothalamic release of CART 4 weeks postinjection. When fed on a normal-chow diet this was associated with a 4% increase in food intake and an increase in body weight gain, an effect which was significant by 64 days postinjection. These differences in food intake and body weight became more marked when the animals were transferred to a high-fat diet, with a 6% increase in food intake and no evidence of escape. rAAV transgene expression has a slow onset (32), but sustained transgene expression has been demonstrated 25 months after gene transfer (20). Gene transfer was confirmed by the detection of WPRE in hypothalamic RNA by RPA. Any animal in which WPRE expression was not detected was excluded from the data analysis (<5%). Furthermore, *in situ* hybridization for WPRE in two representative brains confirmed localization to the PVN. WPRE is part of the rAAV construct, which increases transgene expression but is not normally expressed within the rat central nervous system (28). We have previously demonstrated >80% accuracy in the localization of gene transfer after microinjection into the PVN (33). In addition, we have demonstrated that injection of 1 μ l rAAV-EGFP into the PVN is localized to that nucleus alone (33).

ICV administration of CART (55–102) reduces food intake but results in a distinctive pattern of motor abnormalities which may be due to the activation of hindbrain neuronal circuits. Plugging the cerebral aqueduct between the third and fourth ventricles attenuates both the movement abnormalities and the reduction in food intake observed after ICV

CART (55–102) administration (17). ICV injection of anti-CART anti-serum reduces night time feeding, suggesting CART exerts an endogenous inhibitory tone on food intake (7). CART immunoreactive neurons are found in brainstem nuclei, including the area postrema, the nucleus of the solitary tract (NTS) and the parabrachial nucleus (34). Administration of 0.1 nmol CART (55–102) into the third ventricle or 0.2 nmol into the fourth ventricle, has been shown to activate brainstem neurons, notably in the NTS (5,35,36). It is possible that CART circuits in the hypothalamus and the hindbrain have opposing effects on energy homeostasis and that ICV administration of CART peptides may therefore be activating and ICV CART antibodies blocking, anorectic CART circuits in the hindbrain rather than the hypothalamus (5,7). We have previously shown that acute administration of CART into discrete hypothalamic nuclei increases food intake and does not cause motor or behavioral abnormalities (12). No motor abnormalities were observed in the current study. In accord with these results, long-term intra-ARC administration of CART (55–102) or overexpression of ARC CART using stereotactically targeted gene transfer increases food intake (13).

Intra-PVN injection of rAAV-CART resulted in a small increase in food intake and body weight gain which was significant over the course of the study. Intra-PVN injection of rAAV-CART also resulted in an increase in hypothalamic release of NPY and AgRP at 4 weeks postinjection. It is therefore possible that CART may not directly increase food intake but may act indirectly via the NPY/AgRP neuron. It is unclear the exact mechanism by which CART increases NPY and AgRP release. Within the ARC, the majority of CART neurons are colocalized with POMC, the precursor of the anorexigenic α -MSH (37). POMC-expressing neurons and a second population of neurons which coexpress NPY and AgRP form reciprocal mutual synapses and inhibit each other's activity (27,38–40). CART has been reported to stimulate NPY and AgRP release (9,27). CART may stimulate NPY and AgRP release directly or via other neuronal populations. After intra-PVN rAAV-CART administration, no changes in hypothalamic neuropeptide mRNA expression were observed. It is possible that small changes in hypothalamic NPY or AgRP mRNA in specific nuclei may not have been detected in whole hypothalamic RNA samples.

In conclusion, we have demonstrated that overexpression of CART in the PVN of male rats increases food intake and body weight gain. These results provide further support for the proposal that the hypothalamic CART system has an orexigenic role.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/oby>

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DISCLOSURE

The authors declared no conflict of interest.

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