Melanocortin activation of nucleus of the solitary tract avoids anorectic tachyphylaxis and induces prolonged weight loss

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Li G, Zhang Y, Rodrigues E, Zheng D, Matheny M, Cheng KY, Scarpace PJ. Melanocortin activation of nucleus of the solitary tract avoids anorectic tachyphylaxis and induces prolonged weight loss. Am J Physiol Endocrinol Metab 293: E252–E258, 2007. First published March 27, 2007; doi:10.1152/ajpendo.00451.2006.—To examine the role of the brain stem melanocortin system in long-term energy regulation, we assessed the effects of overproduction of proopiomelanocortin (POMC) in the caudal brain stem of F344xBN rats with adult-onset obesity. Recombinant adeno-associated viral vector encoding POMC gene was delivered to the nucleus of the solitary tract (NTS) in the hindbrain, and food intake, body weight, glucose and fat metabolism, brown adipose tissue thermogenesis, and mRNA levels of neuropeptides and melanocortin receptors were assessed. POMC delivery resulted in sustained reduction in food intake and body weight over 42 days and improved insulin sensitivity. At death, in recombinant adeno-associated viral vector-POMC-treated rats vs. control rats, α-melanocyte-stimulating hormone in NTS increased nearly 21-fold, whereas hypothalamic α-melanocyte-stimulating hormone remained unchanged. Visceral adiposity decreased by 37%; tissue triglyceride content diminished by 26% and 47% in liver and muscle, respectively; serum triglyceride and nonesterified fatty acids were reduced by 35% and 34%, respectively; phosphorylation of acetyl-CoA carboxylase was elevated by 63% in soleus muscle; brown adipose tissue uncoupling protein 1 increased by 30%; and melanocortin 3 receptor expression declined by 60%, whereas melanocortin 4 receptor mRNA levels were unchanged in the NTS. In conclusion, POMC overexpression in the NTS produces a characteristic unabated hypophagia that is uniquely different from the anorexic tachyphylaxis following POMC overexpression in the hypothalamus. The sustained anorectic response may result from absence of compensatory elements in the NTS, such as increased agouti-related protein expression, suggesting melanocortin activation of the brain stem may be a viable strategy to alleviate obesity.

adult-onset obesity; proopiomelanocortin overexpression; gene delivery; brain stem

THE BRAIN MELANOCORTIN PATHWAY is a key leptin target in the central nervous system and plays an essential role in the homeostatic regulation of body weight (6, 8, 28). Melanocortins are peptides cleaved from a common precursor, proopiomelanocortin (POMC). Rodents with POMC deficiency and humans with POMC mutations are hyperphagic and obese (15, 35). The contribution of the central melanocortin system on the regulation of food intake and body weight has been attributed primarily to hypothalamic POMC neurons in the arcuate nucleus (ARC), which produce alpha-melanocyte-stimulating hormone (α-MSH), the principal central melanocortin. α-MSH and its analog, melanotan II (MTII), inhibit food intake and enhance energy expenditure mainly through activation of melanocortin 3 (MC3R) and 4 (MC4R) receptors in the hypothalamus (5). However, the central melanocortin system is not limited to the hypothalamus. POMC neurons and α-MSH are both found within the commissural region of the nucleus of the solitary tract (NTS) in the brain stem (11, 24) where MC4Rs are also expressed (14, 22). Thus melanocortin signaling within the NTS may contribute to, or even play a major role in, the overall central melanocortin system activity. Additionally, because hypothalamic POMC neurons project to the NTS (24, 37), the NTS could serve as a site integrating the neuroendocrine and metabolic impact of POMC, both generated within the NTS and the ARC.

The role of the NTS POMC network in energy homeostasis is just beginning to be appreciated. The functions of melanocortin action in the NTS have been explored by acute administration of pharmacological agents into the fourth ventricle or the dorsal vagal complex (9, 33, 37). These studies indicated that MC4R agonists and antagonists affect food consumption in the caudal brain stem as potently as that in the hypothalamus. In addition, central infusion of MTII into either the third or fourth ventricle also increases brown adipose tissue (BAT) thermogenesis (4, 19, 32). Thus the brain stem melanocortin pathway is responsive to acute pharmacological melanocortin stimulation and likely participates in the regulation of energy balance, possibly in tandem with the melanocortin system in the hypothalamus. Furthermore, the NTS and other brain stem nuclei are generally assumed to respond to short-term signals that regulate meal initiation and termination, whereas those in the ARC and other areas of the hypothalamus predominately respond to long-term adiposity signals (7, 10). However, it remains unknown how chronic activation of the melanocortin system in the caudal brain stem, including the NTS, will affect the overall energy homeostasis.

The F344xBN rats represent an established aging rodent model to investigate adult-onset obesity, which is characterized by a modest progression in body weight and visceral adiposity gain with age (20). Although the aged, obese F344xBN rats maintain their hypothalamic POMC expression with age (36), the induction of POMC by exogenous leptin is impaired, indicating an age-related leptin resistance (27). Our earlier study demonstrated that these animals, albeit leptin resistant,
lost significant amounts of body fat and weight in response to either MTII treatment or viral-mediated POMC gene delivery into the ARC (18, 19, 36). Anorexia induced by either treatment underlies one mechanism for the weight and fat loss. Unfortunately, the reduction in food intake attenuates within days or weeks after the initiation of either therapy, limiting long-term effectiveness of these modalities in treating the adult-onset obesity (18, 19, 36). The rapid attenuation of the anorexic response, or tachyphylaxis, may be a result of dwindling melanocortin receptor function due to reduced receptors (18, 29) and/or increased agouti-related protein (AgRP) antagonism (1). Melanocortin activation in the hypothalamus leads to both increased AgRP expression and reduced MC3R and MC4R expressions (1, 18). However, these responses, especially the former, may be specific to the hypothalamus because AgRP mRNA expression is limited to the ARC (2). Thus it is unclear whether anorexic tachyphylaxis will occur after chronic POMC overexpression in the NTS.

To address these issues, recombinant adeno-associated virus (rAAV) vector encoding murine POMC (rAAV-POMC) was microinjected into the NTS, and the long-term consequences of this POMC gene delivery on energy balance, glucose and fat metabolism, BAT thermogenesis, and mRNA levels of neuropeptides and melanocortin receptors in either the NTS or ARC were assessed.

**MATERIALS AND METHODS**

**Experimental animals.** Male F344xBN rats aged 22 mo were obtained from Harlan Sprague-Dawley (Indianapolis, IN) under contract with the National Institute on Aging. Animals were cared for in accordance with the principles of the National Institutes of Health Guide to the Care and Use of Experimental Animals. Protocols were approved by the University of Florida Animal Care and Use Committee. Rats were housed individually with a 12:12-h light-dark cycle (lights on from 0700 to 1900). Animals had free access to standard Purina 5001 rodent diet and water.

**Description and administration of rAAV-POMC.** rAAV vectors encoding the full-length 935-bp murine POMC cDNA (30) or an enhanced form of green fluorescent protein (GFP; control vector) under the control of the hybrid cytomegalovirus immediate-early enhancer/chicken β-actin promoter were prepared as previously described (18).

Rats were bilaterally administered rAAV-POMC or rAAV-control, 2.51 × 10^10^ particles/injection in 1 μl, into the NTS under inhalation anesthesia (Isoflurane, Baxter, Deerfield, IL). Coordinates were 0 mm from posterior occipital suture, 0.5 mm lateral from the mid sagittal suture, and 10 mm below the skull. Using a Hamilton microsyringe, we delivered a 1-μl volume of virus stocks over 5 min to each site. The coordinates were verified previously in three separate rats by visualization of blue dye in the NTS.

**O2 consumption.** O2 consumption was assessed in up to seven rats simultaneously at days 17 and 25 after gene delivery with an oxygen analyzer (model S-3A; AEI Technologies, Naperville, IL). The rats were placed into the chamber for 60 min with an air flow of 150 ml/min and sampling interval of 8 min without food.

**Tissue harvesting.** Rats were killed by cervical dislocation under pentobarbital sodium anesthesia at day 42. Cardiac blood, hypothalamus, BAT, and perirenal (PWAT), retroperitoneal, and epididymal white adipose tissues, liver, and soleus muscle were obtained as anterior commissure for ARC, corresponding to the brain atlas of Paxinos and Watson (25). The NTS and a block of the neighboring reticular nucleus (as an outside NTS control) were removed from the 2-mm-thick slice, and the ARC was removed from the 3-mm-thick slice by a Stoelting Brain Punch Set. The brain tissue samples were boiled and sonicated in 0.5 ml of 0.1 M acetic acid. Homogenates were centrifuged (13,000 g) for 15 min. To assay for protein, 60 μl of supernatant were taken from each sample, and the remainder was stored at −80°C until RIA analysis for α-MSH.

**Western analysis.** Uncoupling protein 1 (UCP1) in BAT homogenates was measured with anti-human UCP1 antibody (Linco Research, St. Charles, MO) (16). To determine the phosphorylated acetyl-CoA carboxylase (ACC) and total ACC in liver, soleus muscle, and PWAT, the protein homogenates (20–50 μg) were assessed with either a monoclonal antibody specific to phosphorylated ACC (Upstate Cell Signaling Solutions, Lake Placid, NY) or a streptavidin-horseradish peroxidase-conjugated antibody (at 1:10,000 dilution) specific for the biotin-associated total ACC (Pierce Biotechnology, Rockford, IL) and visualized by enhanced chemiluminescent detection (ECL-plus, Amersham Pharmacia Biotech, Piscataway, NJ).

**Serum hormones and metabolites and tissue α-MSH levels.** Serum leptin was measured with a Linco rat RIA kit. Serum nonesterified fatty acids (NEFA) and triglyceride were determined by enzymatic colorimetric kits (Wako Chemicals, Richmond, VA). Tissue α-MSH levels were assessed by an RIA kit (Phoenix Pharmaceuticals, Mountain View, CA).

**Triglyceride contents of liver and soleus muscle.** Liver and soleus muscle were homogenized, and triglyceride was extracted with chloroform-methanol 2:1 (vol/vol), dried, and resuspended in 100% ethanol. Triglyceride contents were measured with a Wako L-type TG-H kit.

**RT-PCR.** Expression levels of POMC, neuropeptide Y (NPY), AgRP, MC3R, and MC4R in the hypothalamus and NTS were identified by relative quantitative RT-PCR with the QuantumRNA 18S internal standards kit (Ambion, Austin, TX) (16, 17, 19). PCR was performed by multiplexing corresponding primers (POMC sense 5′-GCTGCAACCTGACCTTCTC-3′, antisense 5′-CCGGACGGTCTTGA-3′; NPY sense 5′-ATGGGGCTGTGGAGGATCGACC-3′; antisense 5′-GTCAAGAGACAGITTATCTTT-3′; AgRP sense 5′-AGGGATCGAAGGCTTGCAA-3′, antisense 5′-GGCCACGATCCAGGAG-3′; and MC4R sense 5′-AGTCTCTGGAGAAGGCGGCGA-3′, antisense 5′-CACGTAGTATGAGCTGCCAG-3′). 18S primers, and competitors and coamplifying. The optimum ratio of 18S primer to competitor was 1:5 (hypothalamus and NTS) for POMC, 1:7 (hypothalamus) and 1:9 (NTS) for NPY, 1:4 (hypothalamus) and 1:9 (NTS) for AgRP, 1:6 (hypothalamus) and 1:9 (NTS) for MC3R, and 1:9 (hypothalamus and NTS) for MC4R. PCR was performed at 94°C denaturation for 60 s, 59°C annealing temperature for 50 s, and 72°C elongation temperature for 50 s for 26 cycles, 22 cycles, or 28 cycles (18S). Primers were designed to detect the corresponding amplicon by 10.220.33.1 on October 14, 2017 http://ajpendo.physiology.org/ Downloaded from
RESULTS

POMC expression and α-MSH production in the NTS of rats with adult-onset obesity. The POMC transgene overexpression was verified by RT-PCR using brain micropunch samples (Fig. 1, top). Forty-two days after vector delivery, NTS POMC mRNA levels were elevated over sevenfold in rAAV-POMC rats compared with controls (**P < 0.001). In contrast, POMC mRNA was not elevated in the hypothalamus after POMC gene delivery into the NTS. Concentrations of α-MSH were also assessed in control and rAAV-POMC-administered rats (Fig. 1, bottom). In control rats, basal α-MSH levels in the ARC were 11 times greater than in the NTS. However, after rAAV-POMC NTS gene delivery, there was a 21-fold increase in levels of α-MSH in the NTS but no change in the ARC. In addition, there was an apparent sixfold elevation of α-MSH in the neighboring reticular nucleus over the baseline α-MSH level in the NTS. In a separate group of rAAV-GFP-administered rats, the GFP staining pattern was examined with a specific antibody to GFP in the brain stem slice obtained from coronal section between Bregma −13.24 to −13.34 mm. GFP-positive cells were distributed diffusely in the NTS and surrounding nuclei, including central, dorsolateral, intermedial, medial, ventral, ventrolateral, and para solitary tract. The diffused GFP staining pattern agrees well with the nature of intermixing and loose organization of NTS neurons. No GFP immunofluorescence was found in the hypothalamic brain slices (data not shown).

Food consumption and body weight. After rAAV-POMC administration into the NTS, food consumption decreased rapidly and became significantly different from control rats by day 3 (Fig. 2, top). Between days 3 and 7, the reduction in food intake reached a nadir, amounting to a 33% reduction compared with rats administered control vector (Fig. 2, top). Starting at day 8, the anorexic response began to wane, yet food consumption remained diminished by >3 g/day throughout the duration of the experiment (Fig. 2, top).

Before vector delivery, average body weight of rAAV-POMC-treated rats was comparable to that of rAAV-control rats (585 ± 11 vs. 590 ± 6 g, respectively, at day 0). Immediately after vector delivery, both POMC and control rats lost ~20 g of body weight. This is what we normally observe after surgery in aged, obese rats (18, 27). Whereas body weight of rAAV-control rats remained steady throughout the experimental period, there was a steady decrease in body weight during the first 30 days after rAAV-POMC gene delivery (Fig. 2, bottom). After day 30, body weight stabilized in these rats despite the persistent reduction in food consumption (Fig. 2, bottom). At the end of the experiment (day 42), rAAV-POMC
rAAV-POMC-treated rats had lost an average of 72 ± 6 g of body weight compared with 29 ± 5 g in control rats (P < 0.001).

Adiposity and serum leptin levels. The decrease in body weight with rAAV-POMC delivery was associated with diminished adiposity levels. Forty-two days after central POMC gene delivery, there was a >37% reduction in visceral adiposity, as reflected by the sum of the PWAT and retroperitoneal white adipose tissues in rAAV-POMC-treated vs. in control rats (Fig. 3, top). In addition, epididymal adipose tissue was diminished by 31% with POMC overexpression (Fig. 3, top). Given the difference in overall body weight, we also normalized the sum of the three fat depots to total body weight. By this calculation, visceral adiposity was also significantly reduced with POMC treatment relative to controls (3.52 ± 0.33% of total body weight vs. 4.89 ± 0.17%; P < 0.005). Serum leptin levels, another indicator of body fat mass, were 38% lower in the POMC group vs. controls (Fig. 3, bottom).

Fasting insulin and glucose. Fasting glucose and insulin were determined on day 29 after POMC gene delivery (Table 1). Whereas POMC treatment did not alter fasting glucose, fasting insulin levels were diminished by >60%. Calculation of the quantitative insulin sensitivity check index (12) revealed that the rAAV-POMC-treated rats had increased insulin sensitivity.

Energy expenditure. Energy expenditure after rAAV-POMC was assessed as whole body oxygen consumption and UCP1 protein levels in BAT. Oxygen consumption was recorded at day 17 and day 25 after vector delivery. On both of these days, oxygen consumption, whether expressed as consumption per rat or normalized to body weight, was not different between rAAV-POMC-treated and control rats (data not shown). However, because food intake was still depressed in the rAAV-POMC-treated rats, it was possible that any POMC-induced increase in oxygen consumption was masked by the suppression in energy expenditure due to the diminished food intake. For this reason, we also assessed UCP1 protein levels in BAT at the termination of the experiment (Table 2). Induction of UCP1 in BAT is an important marker for enhanced thermogenesis and thus energy expenditure in rodents (3). The activation of BAT by leptin or MTII is normally associated with an increase in UCP1 and a decline in BAT tissue mass due to the lipolysis associated with thermogenesis (19, 27). In the present study, total BAT weight declined markedly with rAAV-POMC treatment, and UCP1 protein concentration was elevated by 30%. However, there was only a mild increase in total UCP1 protein per BAT (Table 2).

Phosphorylation of ACC. Inactivation of ACC by phosphorylation is one indicator of augmented fatty acid oxidation and/or diminished fatty acid synthesis (13). We examined phosphorylation of ACC 42 days after POMC gene delivery in three tissues: soleus muscle, PWAT, and liver (Fig. 4). In soleus muscle, POMC treatment increased ACC phosphorylation by 63%. On the contrary, in PWAT, phosphorylation of ACC was diminished by nearly 60%. This decrease in phosphorylated ACC was accompanied by a 40% decrease in total ACC (100 ± 11 vs. 63 ± 12 arbitrary units/mg protein, respectively, for POMC treatment and control; P = 0.047). In

Table 1. Fasting glucose, fasting insulin, and insulin sensitivity indexes 29 days after rAAV-POMC or rAAV-control delivery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Glucose-to-insulin ratio</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rAAV-Control</td>
<td>79 ± 5</td>
<td>2.2 ± 0.4</td>
<td>42 ± 8</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>rAAV-POMC</td>
<td>70 ± 6</td>
<td>0.8 ± 0.2*</td>
<td>98 ± 19*</td>
<td>0.59 ± 0.03*</td>
</tr>
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Values are means ± SE of 6 or 7 rats per group. rAAV, recombinant adenoviral vector; POMC, proopiomelanocortin; QUICKI, quantitative insulin sensitivity check index, calculated as 1/[log(I) + log(G)], where I and G are fasting insulin and glucose levels, respectively. *P < 0.05 and †P < 0.01 for difference of POMC treatment vs. control by unpaired t-test.
liver, phosphorylation of ACC was unchanged with POMC gene delivery.

**Triglyceride and NEFA.** At the termination of the experiment, we assessed triglyceride levels in serum, liver, and muscle as well as NEFA level in serum (Table 3). Triglyceride levels were significantly diminished by 26% in liver and by 35% in serum. There was also a trend toward a decrease in triglyceride level in skeletal muscle without significance. Parallel to the decrease in serum triglyceride, NEFA serum level was diminished by 34% (Table 3).

NPY, AgRP, MC3R, and MC4R mRNA levels. The effects of POMC gene delivery on the expression of neuropeptides and melanocortin receptors in the NTS were measured 42 days after POMC vector delivery. RT-PCR revealed that the expression of AgRP, the endogenous antagonist of melanocortin receptors, and those of NPY were unchanged in rats given rAAV-POMC compared with control rats in the NTS (Table 4). In addition, the level of AgRP mRNA in the hypothalamus was found to be unchanged (0.66 ± 0.01 vs. 0.65 ± 0.03, respectively, for control and rAAV-POMC treatment; P = 0.66). However, it is noteworthy that this basal AgRP expression in the hypothalamus is far greater than the basal AgRP expression in the NTS. For example, when amplified under the same PCR conditions for hypothalamic AgRP, NTS AgRP was undetectable (data not shown).

MC3R and MC4R are believed to be the predominant melanocortin receptors in the NTS that mediate the effects of POMC-derived α-MSH on the homeostatic regulation of body weight. Whereas the expression level of MC4R in NTS was unchanged, that of MC3R was reduced by nearly 60% in rAAV-POMC-treated compared with control rats (Table 4).

**DISCUSSION**

With the use of neural site-directed rAAV-mediated POMC gene delivery, POMC overexpression and increased α-MSH production were observed in the NTS area at 42 days after vector delivery. Conversely, neither POMC expression nor α-MSH levels were elevated in the hypothalamus.

The chronic POMC overexpression in the NTS causes persistent but moderate anorexia, a pattern in sharp contrast to pharmacological α-MSH or MTII administration into the third or MTII administration into the fourth ventricle, which induces a suppression in caloric intake for only a few days in either normal or dietary obese mice and rats (9, 21, 26). The sustained anorexic response in the present study is also considerably longer than the 20 days of anorexia observed after rAAV-POMC gene delivery targeted to the ARC of the hypothalamus in the age-matched rats of the same strain (18).

The mechanism of the rapid tachyphylaxis to melanocortin treatment in pharmacological studies is not clear but may involve agonist-mediated receptor internalization (18, 29) and/or elevated AgRP levels (1). MC3R and MC4R activation by α-MSH in the hypothalamus is subject to competitive suppression by the natural antagonist, AgRP (8). Because hypothalamic AgRP expression rises after either peripheral MTII application (1) or hypothalamic POMC gene delivery in young rats (our unpublished data), AgRP seems to be a good candidate for mediating the anorexic tachyphylaxis. Expression of AgRP mRNA is abundant in the ARC (2), and AgRP-containing neurons in the ARC project to other neurons in the hypothalamus and brain stem (34). However, immunohistochemical analysis identifies few AgRP-positive neurons in the NTS (2). In the present study, the expression of AgRP remains unchanged in both the NTS and ARC after POMC gene delivery into the NTS, and the basal level of AgRP in the NTS dwarfs that in the ARC. Thus the lack of AgRP antagonism in the NTS may be one factor preserving the anorectic response to POMC overexpression in the NTS. Another factor may involve MC3R and MC4R expressions. Our previous study with POMC gene delivery into the hypothalamus demonstrated diminished expression levels of MC3R and MC4R in the
hypothesis (18). On the contrary, only the expression level of MC3R was decreased in the present study, whereas MC4R expression level was unchanged. This absence of a downregulation of MC4R expression in the NTS may also contribute to the prolonged anorexia. The rAAV-mediated POMC overexpression in the NTS did result in the elevation of α-MSH peptide levels outside the NTS; therefore, the ectopic expression of POMC in the caudal brain might account for some of the responses observed.

POMC gene delivery into the NTS leads to a significant decrease in visceral adiposity and a sustained reduction in body weight in rats with adult-onset obesity. Body weight commenced to decline within days of rAAV-POMC gene delivery and continued for 30 days, after which body weight stabilized. The incongruent patterns of food intake and weight loss suggest factors other than just food intake contributed to the reduced body weight. The anorectic response displayed three phases: a peak response between days 3 to 8, a partial recovery period between days 8 and 14, and a prolonged, moderate anorexia between days 15 and 42. The body weight response, on the other hand, demonstrated a steady, almost linear decrease in body weight from day 3 to day 30, after which it stabilized. This discrepancy suggests that, in addition to the diminished food intake, increased energy expenditure may facilitate the weight and fat reduction. This notion is supported by elevated UCP1 protein levels. Albeit modest, the augmented UCP1 is indicative of an increase in BAT thermogenesis after POMC gene delivery to the NTS. Inconsistent with these observations is the lack of an increase in whole body oxygen consumption. Conclusive assessments of any increase in energy expenditure and any food-independent component of the body weight loss cannot be determined from these studies and would require the inclusion of a pair-fed group.

In addition to the reduction in body weight, there was a substantial decrease in adiposity levels and triglyceride levels and an apparent increase in fat oxidation in muscle. These may be a direct result of enhanced energy expenditure or a consequence of chronic anorexia or both. The evidence for augmented fat oxidation was an increase in phosphorylation of ACC, a key enzyme in regulation of fat oxidation in muscle (13). Phosphorylation of ACC inactivates the enzyme, thus reducing the synthesis of malonyl CoA. A reduction in the latter releases the inhibition of carnitine palmitoyl transferase-I, the activity of which is the rate-limiting step in muscle mitochondrial fat oxidation. Despite the apparent increase in fat oxidation in muscle, phosphorylation of ACC was not elevated in liver and was diminished in PWAT. The latter is inconsistent with what is observed after chronic leptin treatment in young, lean rats, which substantially increase fat oxidation within the fat tissue (31). The pattern observed in the present study, an increase in fat oxidation in muscle and reduced fat metabolism in fat tissue, seems to agree with what would be expected after chronic food restriction. In such situations, the stored fat would be used as necessary fuel (oxidation in muscle) and not for facilitated thermogenesis (oxidation in BAT or white adipose tissue). Conceivably, the chronic anorexia is the primary cause of the apparent increase in fat oxidation in muscle rather than an overt increase in energy expenditure.

The NTS POMC overexpression also improved insulin sensitivity. The aged F344xBN rats with adult-onset obesity normally demonstrate insulin resistance and glucose intolerance (18). The fasting insulin levels were substantially diminished in the POMC-treated rats, and the Quantitative Insulin Sensitivity Check Index (12) indicated increased insulin sensitivity. These data are consistent with our previous report of improved glucose metabolism and insulin sensitivity after POMC gene delivery into the hypothalamus and in agreement with other findings that central melanocortin receptor activation suppresses insulin release from the pancreas and enhances glucose metabolism (8, 17, 18, 23). The enhanced insulin sensitivity is probably due to the substantial decrease in visceral adiposity and muscle triglyceride content by the chronic POMC treatment and could be the result of the prolonged anorexia and/or some food-independent function(s) specific to POMC overexpression.

In conclusion, POMC gene delivery directed into the NTS suppresses food intake, reduces body weight and visceral adiposity, increases muscle fat oxidation and BAT UCP1 protein levels, lowers tissue triglyceride content, and improves insulin sensitivity in rats with adult-onset obesity. The unabated hypophagia, unique to POMC overexpression in the NTS compared with in the hypothalamus, suggests that the mechanisms leading to anorectic tachyphylaxis in response to melanocortin activation in the hypothalamus are lacking in the NTS. Therefore, rAAV-POMC gene delivery to the NTS appears more efficacious than comparable activation in the hypothalamus and is a new and viable strategy to combat adult-onset obesity in rodents.

GRANTS

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POMC OVEREXPRESSION IN NTS AMELIORATES OBESITY