SUPPLEMENTARY DATA

Immunohistochemistry

MSG lesions were checked by NPY immunohistochemistry and compared to saline infused rats. For immunohistochemical staining, animals were injected with pentobarbital IV and perfused intra-arterially with saline and 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). After post-fixation and equilibration for 48 hrs in 30% sucrose in 0.1M Tris-buffered saline (TBS), the brain tissue was cut on a cryostat into 35 μm sections into 4 groups. Free floating sections were incubated in the primary antiserum rabbit anti-NPY (1: 1,000) overnight at room temperature. The NPY antibody (Niepke 26/11/1988, Netherlands Institute for Brain Research) was raised in rabbit against porcine NPY (N4509; Sigma) as previously described (Buijs et al. 1989; van der Beek et al. 1992; Goldstone et al. 2002). Sections were rinsed in TBS and incubated in biotinylated goat anti-rabbit antibody (1:800; Vector Laboratories, Burlingame, CA) for 1 h, rinsed three times in TBS, and incubated in avidin–biotin complex (Elite ABC, Vector Laboratories) for 1 h. After three rinses, sections were incubated in 1% diaminobenzidine (DAB) and 0.05% nickel ammonium sulfate and reacted with 0.01% hydrogen peroxide. Sections were washed, mounted on gelatin-coated glass slides, dehydrated in graded alcohols, cleared in xylene, and coverslipped using Entellan for observation by light microscopy.

Buijs RM, Pool, CW, Heerikhuize JJ, van Sluiter AA, van der Sluis PJ, Ramkema M, van der Woude TP, van der Beek E. Antibodies to small transmitter molecules and peptides: production and application of antibodies to dopamine, serotonin, GABA, vasopressin, vasoactive intestinal peptide, neuropeptide Y, somatostatin and substance P. Biomedical research 1989;10:213-221

van der Beek EM, Pool CW, van Eerdenburg FJ, Sluiter AA, van der Donk HA, van den Hurk R, Wiegant VM. Fc-mediated nonspecific staining of the porcine brain with rabbit antisera in immunocytochemistry is prevented by pre-incubation of the sera with proteins A and G. J Histochem Cytochem 1992;40:1731-1739

Goldstone AP, Unmehopa UA, Bloom SR, Swaab DF. Hypothalamic NPY and agouti-related protein are increased in human illness but not in Prader-Willi syndrome and other obese subjects. J Clin Endocrinol Metab 2002;87:927-937

Western Blot analysis.

A portion of liver tissue was homogenized in 1 ml buffer (0.05 M sodium phosphate, 2 mM EDTA, 25 mM DTT, 125 mM sucrose, 5% glycerol, 1 mM PMSF, peroxovanadate and protease inhibitors (Complete, Roche). Homogenates were subjected to centrifugation and total protein was determined in supernatant using a commercially available kit (Bio-Rad, Hercules, USA). Equal amounts of protein were diluted in Laemmli sample buffer. The samples were boiled for 5 minutes. 30 μg of protein was loaded on a 10% SDS-PAGE gel. Gels were blotted on Immobilon-P Transfer Membrane (Millipore, Bedford, USA). Phosphorylation and levels of several proteins were determined using the following antibodies: ACC (#3662, Cell Signalling Technology), phosphorylated ACC (Ser 79, #3661, Cell Signalling Technology) and rabbit-anti-goat-HRP (P0449, DAKO). All membranes were normalized with Actin (I-19, sc-1616, Santa Cruz). Bound antibodies were visualized by enhanced chemiluminescence and quantified by densitometry analysis of scanned images (LumiImager, Boehringer, Mannheim).

SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences used for real-time PCR.

Gene	Accesion no.	Forward sequence	Reverse sequence	Product	Annealing T
ACC1	NM_022193	GATGATCAAGGCCAGCTTGT	CAGGCTACCATGCCAATCTC	68	60
ACC2	NM_053922	GACGAAGGCGAGTCAGGTAT	GAAGCCTCTCCTGCAATCAT	101	55
FAS	NM_017332	CTTGGGTGCCGATTACAACC	GCCCTCCCGTACACTCACTC	185	57
CPT1α	NM_031559	ACAATGGGACATTCCAGGAG	AAAGACTGGCGCTGCTCA	65	55
PPARγ	NM_001145366	CAGGAAAGACAACAGACAAATCA	GGGGGTGATATGTTTGAACTTG	95	55
SREBP1c	XM_213329	ACAAGATTGTGGAGCTCAAGG	TGCGCAAGACAGCAGATTTA	72	60
SCD1	NM_139192	CTACAAGCCTGGCCTCCTGC	GGCACCCAGGGAAACCAGGA	226	60
MTTP	NM_001107727	GCGAGTCTAAAACCCGAGTG	CACTGTGATGTCGCTGGTTATT	62	55
ApoB	NM_019287	CACCTAAGATCAACAGTCGCTTC	TCGAAAGCCAGACCCACTT	61	55
ARF-1	NM_022518	CGGAACCAGAAGTGAACCAGACC	CGTTTGCCACATGAGAGGAAAGC	81	55

PCR product length (bp), Annealing temperature (°C).