Physiology & Behavior, Vol. 67, No. 4, pp. 521–525, 1999

0031-9384/99/$–see front matter

PII S0031-9384(99)00095-5

Neuropeptide Y Overexpression in the Preweanling Zucker (*fa/fa*) Rat

TIMOTHY J. KOWALSKI,*[1] THOMAS A. HOUPT,* JEONGWON JAHNG,† NORI OKADA,‡ SHUN-MEI LIU,‡ STREAMSON C. CHUA, JR.‡ AND GERARD P. SMITH*

**E. W. Bourne Laboratory, Department of Psychiatry, Weill Medical College of Cornell University and New York Presbyterian Hospital, White Plains, NY 10605; †Laboratory of Molecular Neurobiology, Burke Medical Research Institute, Department of Neurology and Neuroscience, Weill Medical College of Cornell University, White Plains, NY 10605; and ‡Division of Molecular Genetics, Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, NY 10032*

Received 19 April 1999; Accepted 29 April 1999

KOWALSKI, T. J., T. A. HOUPT, J. JAHNG, N. OKADA, S.-M. LIU, S. C. CHUA AND G. P. SMITH. *Neuropeptide Y overexpression in the preweanling Zucker* (fa/fa) *rat.* PHYSIOL BEHAV **67**(4) 521–525, 1999.—Hypothalamic preproNPY overexpression in the Zucker fatty (*fa/fa*) rat was examined. In situ hybridization was used to determine the relative level of preproNPY mRNA in the arcuate nucleus of +/+, +/*fa*, and *fa/fa* pups aged postnatal day 2 (P2), 5, 9, 12, or 25. The relative optical density (ROD) of probe hybridization in the arcuate, the area of hybridization (A), and the product of ROD × A (a measure of total arcuate preproNPY mRNA hybridization) were measured. Values were normalized to the mean +/*fa* value within each litter. Initial analysis showed that preproNPY mRNA hybridization (ROD × A) in *fa/fa* pups was significantly higher than +/*fa* and +/+ pups on P9, 12, and 25, and significantly higher than +/*fa* on P5. No significant difference between lean (+/+ and +/*fa*) genotypes, however, were observed at any age tested. Values from the lean genotypes were, therefore, pooled, and data were normalized to the mean value of lean animals for analysis. This analysis revealed that preproNPY mRNA hybridization in *fa/fa* pups was higher than lean littermates as early as P2. © 1999 Elsevier Science Inc.

Genetic obesity Arcuate nucleus Hypothalamus Development of obesity

THE Zucker fatty rat (*fa/fa*) is a model of genetic obesity with an autosomal recessive mode of inheritance (42). *fa/fa* rats display a juvenile-onset obesity with accompanying metabolic and behavioral abnormalities, such as hyperphagia, decreased energy expenditure, hyperinsulinemia, and hypercoticosteronemia (7). The *fa* mutation has recently been identified as an amino acid substitution in the extracellular domain of the receptor for leptin (10,11). All leptin receptor isoforms are affected by this mutation. As a consequence, the number of receptors at the cell surface (27), and their signal transduction efficiency (40), are decreased. Additionally, *fa/fa* rats have elevated plasma leptin levels (39), and are resistant to exogenous leptin administration (13).

Studies comparing adult *fa/fa* and +/? rats have shown that *fa/fa* rats have higher preproneuropeptide Y (preproNPY) mRNA in the arcuate nucleus of the hypothalamus (30), higher levels of NPY peptide in several hypothalamic nuclei (4), and higher rates of NPY release into the PVN (15). Hypothalamic NPY activity is elevated in other models of obesity (37,38), and chronic intracerebroventricular administration of NPY mimics many of the physiological and behavioral symptoms present in obesity (41), suggesting that increased hypothalamic NPY activity is involved in the etiology of obesity and its associated metabolic and behavioral disturbances.

NPY is an orexigenic peptide when administered centrally, and is most potent when injected into the paraventricular nucleus of the hypothalamus (PVN) (34). The arcuate nucleus is the primary site of NPY synthesis in the hypothalamus (1), and an arcuo-paraventricular NPYergic projection exists (2). This arcuo-paraventricular NPYergic projection is believed to play a role in feeding behavior because its activity is correlated with deprivation state; arcuate preproNPY mRNA, PVN immunoreactive NPY, and release of NPY into the PVN all increase during periods of food deprivation and return to basal levels with refeeding (6,21,28). Additionally, exogenous central NPY administration decreases sympathetic drive to

[1]To whom requests for reprints should be addressed at Columbia University, Division of Molecular Genetics, Department of Pediatrics, 1150 St. Nicholas Ave., New York, NY 10032. E-mail: tjk22@columbia.edu

brown adipose tissue (decreasing thermogenesis) (5), increases plasma insulin levels (25), and stimulates white adipose tissue lipoprotein lipase activity (5).

Recent work has shown that central leptin administration to deprived rats or to *ob/ob* mice (that lack leptin) decreases arcuate preproNPY mRNA expression (31,32). This has led to the hypothesis that the inability of *fa/fa* rats to respond appropriately to circulating leptin results in increased arcuate preproNPY expression with subsequent hyperphagia and decreased energy expenditure (36). It is implicit within this hypothesis that the elevation in hypothalamic NPY expression occurs prior to or simultaneously with the emergence of hyperphagia and decreased energy expenditure. Decreased energy expenditure in *fa/fa* rats has been observed as early as P2 (26) and hyperphagia at P12 (23). The onset of arcuate preproNPY overexpression in *fa/fa* rats, however, has been less clearly defined, varying from P6 to after week 11 (12,20). The purpose of this study was to identify the age that preproNPY overexpression occurs in *fa/fa* rats more precisely. Our results indicate that arcuate nucleus preproNPY mRNA levels in *fa/fa* pups are higher than lean pups as early as P2.

MATERIALS AND METHODS

Animals

Lean (+/+ and +/*fa*) and obese (*fa/fa*) Zucker rats aged P2, 5, 9, 12, and 25 were used in the experiments. Animals were the progeny of primiparous and multiparous +/*fa* females mated with +/*fa* males. All breeders were derived from the Vassar College colony (Poughkeepsie, NY). Mating pairs and pregnant females were housed in Plexiglas containers with wood shavings as bedding. Rats received pelleted chow and water ad lib, and were maintained on a 12:12 h light:dark cycle (0700–1900 H) at 22 ± 2°C. Pregnant females were checked daily for pups, and the day pups were first seen was termed P0. On P3 or P4, animals assigned for study on P9, 12 or, 25 were earclipped for identification and tissue collection (for genotyping; see below). Pups studied on P2 or P5 were marked for identification using a dorsal subcutaneous injection of ink, and spleen was collected for genotyping upon death. A total of 116 pups (+/+ $n = 24$; +/*fa* $n = 56$; *fa/fa* $n = 36$) were used, with 27 pups tested on P2, 23 on P5, 25 on P9, 17 on P12, and 24 on P25. Four to seven litters consisting of 7 to 12 pups were used at each age (not all pups within each litter were used for this experiment). All of the animals from each litter tested were sacrificed on the same day. Data from 45 lean Zucker pups aged P2, 5, 9, and 12 used in the calculations has been previously reported (22).

Tissue Preparation

Between 0700 to 1200 h, animals were individually removed from the dam, weighed, and given an overdose of sodium pentobarbitol i.p. When unresponsive, animals were transcardially perfused with 0.9% NaCl, 0.5% $NaNO_2$, 100 U/mL heparin followed by ice-cold 4% buffered paraformaldehyde (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Brains were removed, postfixed for 24 h in 4% buffered paraformaldehyde and cryoprotected in 30% sucrose at 4°C for 48 h before sectioning.

In Situ Hybridization

Forty-micron frontal sections were cut using a sliding microtome and placed into ice-cold 2× SSC (SSC = 0.15 M NaCl/0.015 M Na Citrate). Six to 10 sections through the mid-region of the arcuate were taken. Tissues were prehybridized in glass vials in 1 mL of 60% formamide, 0.02 M Tris pH 7.4, 1 mM EDTA, 10% dextran sulfate, 0.8% Ficoll, 0.8% PVP, 0.8% BSA, 2× SSC, 0.1 M dithiothreitol, and 1.6 mg/mL herring sperm DNA for 2 h at 48°C. After 2 h, radiolabeled probe was added ($\sim 1.0 \times 10^7$ cpm/vial) and incubated for 16–20 h at 48°C. Hybridization was performed using a 511 base pair (bp) α-[^{35}S]dATP random prime labeled cDNA encoding preproNPY (19). Sections were then sequentially rinsed in 2×, 1×, 0.5×, 0.25×, and 0.125× SSC for 15 min at 48°C and placed into 0.1 M phosphate buffer for mounting. The tissue sections were mounted onto gelatin-coated slides, air dried, and apposed to X-ray film (β-max, Amersham) for 12–24 h. Each litter was processed together, and slides were exposed to the same film. Slides were then dipped into photoemulsion (Kodak) and exposed at 4°C for 1 week for histological verification of probe labeling.

Probe hybridization in the arcuate was quantified by densitometry of autoradiograms using the MCID system (Imaging Research, Inc.; St. Catherines, Canada). The relative optical density of hybridization signal in the arcuate (ROD), the area of hybridization in the arcuate (A), and the product of ROD and A (ROD × A; a measure of total hybridization in the arcuate) were determined from 6–10 sections/animal. The mean ROD, A, and ROD × A values from each animal were used in the calculations (see below).

Determination of Genotype at Lepr

Genotypes were determined according to a previously described method (11). Briefly, the A to C mutation at nucleotide 880 of *Lepr*^{fa} introduces an Msp1 restriction site, which can be used to detect the number of copies of the *Lepr*^{fa} allele. Tissue was digested with proteinase K and genomic DNA was extracted using the Quiagen QIAamp Tissue Kit. Primers: (5′ TGAAGCCCGATCCACCGCTGG 3′ and 5′ CTCTCTTACGATTGTAGAATTCTC 3′) were used to generate a 143 base pair polymerase chain reaction (PCR) fragment. PCR was performed on a Perkin–Elmer DNA Thermal Cycler using the following conditions: 92°C (2 min), 1 cycle; 92°C (30 s), 55°C (30 s), 72°C (1 min), 35 cycles; and 72°C (5 min), 1 cycle. The Advantage Genomic PCR Kit (Clontech) was used for PCR reactions. The PCR fragments were digested with Msp1 (80 mU/μL final concentration) for 1.5 h at 37°C and electrophoresed on a 2% agarose–2% low melting point agarose gel. Digestion yields an uncut 143 bp product for the wild-type allele, while the mutant allele (*fa*) yields both a 106 bp and a 37 bp product.

Statistical Analysis

Results are presented as means ± SEM. The ROD, A, and ROD × A measures were normalized to the mean of heterozygotes within each litter. Heterozygotes were chosen because they were the most numerous. Significant differences in preproNPY mRNA levels among genotypes were initially assessed by a Kruskal–Wallis *H*-test. Post hoc analyses were performed using a Mann–Whitney *U*-test where appropriate. No significant differences between +/+ and +/*fa* (lean) pups were observed at any age tested. In a subsequent analysis, therefore, lean animals were pooled and the ROD, A, and ROD × A of all animals were normalized to lean animals within each litter. Significant differences between *fa/fa* and lean animals were assessed at each age using a *U*-test. An *H*-test was used to evaluate the magnitude of *fa/fa* overexpression among ages. A p-value <0.05 was considered significant.

TABLE 1

BODY WEIGHT AND NORMALIZED RELATIVE OPTICAL DENSITY (ROD), AREA (A), AND ROD × A IN PREWEANLING LEAN (+/+, +/*fa*) AND OBESE (*fa/fa*) ZUCKER RATS

Age	Genotype (*n*)	Body Weight (g)	Normalized* ROD	Normalized* Area	Normalized* ROD × A
P2	*fa/fa* (8)	5.58 ± 0.23	1.10 ± 0.05	1.18 ± 0.04†	1.27 ± 0.07†
	+/*fa* (13)	5.84 ± 0.15	1.00 ± 0.04	1.00 ± 0.05	1.00 ± 0.08
	+/+ (6)	5.66 ± 0.15	1.04 ± 0.08	1.10 ± 0.09	1.16 ± 0.17
P5	*fa/fa* (8)	9.26 ± 0.63	1.09 ± 0.05	1.22 ± 0.08†	1.32 ± 0.07†
	+/*fa* (9)	9.16 ± 0.47	1.00 ± 0.03	1.00 ± 0.04	1.00 ± 0.07
	+/+ (6)	9.00 ± 0.52	1.04 ± 0.02	1.13 ± 0.12	1.18 ± 0.13
P9	*fa/fa* (7)	17.05 ± 0.70	1.18 ± 0.04‡	1.09 ± 0.08	1.29 ± 0.12‡
	+/*fa* (12)	16.90 ± 0.65	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.03
	+/+ (6)	18.10 ± 0.79	0.95 ± 0.03	0.95 ± 0.08	0.91 ± 0.09
P12	*fa/fa* (5)	26.6 ± 4.0	1.28 ± 0.09†	1.22 ± 0.09§	1.60 ± 0.15†
	+/*fa* (9)	24.0 ± 1.2	1.00 ± 0.04	1.00 ± 0.07	1.00 ± 0.10
	+/+ (3)	28.4 ± 2.8	1.10 ± 0.10	0.91 ± 0.05	0.99 ± 0.13
P25	*fa/fa* (8)	51.9 ± 5.6	1.37 ± 0.05‡	1.30 ± 0.06‡	1.78 ± 0.13‡
	+/*fa* (13)	49.8 ± 3.7	1.00 ± 0.01	1.00 ± 0.03	1.00 ± 0.03
	+/+ (3)	43.7 ± 1.7	0.98 ± 0.03	0.91 ± 0.09	0.89 ± 0.09

*Relative optical density (ROD) and area of hybridizaition (A) values were normalized to +/*fa* littermates.
†Significantly different from +/*fa* ($P < 0.05$).
‡Significantly different from +/+ and +/*fa* ($p < 0.05$).
§Significantly different from +/+ ($p < 0.05$).

RESULTS

PreproNPY mRNA levels in the arcuate nucleus (expressed as ROD × A) of *fa/fa* pups were higher than those in +/+ and +/*fa* pups as early as P9 (Table 1). On P5, *fa/fa* pup preproNPY mRNA levels were higher than their +/*fa* littermates but not +/+. No differences between lean pups were observed at any age. When the data from lean animals within each age group were pooled for comparison to *fa/fa* pups, preproNPY mRNA levels (ROD × A) of *fa/fa* pups were higher on P2 P9, 12, and 25, with a trend towards overexpression on P5 ($p = 0.057$) (Fig. 1).

On P2, the significantly higher ROD × A observed in *fa/fa* animals was due to a significantly larger A, whereas on P9 it was due to a higher ROD. On P12 and 25, the higher ROD × A was due to an increase in both ROD and A. There was a significant effect of age on the magnitude of overexpression (ROD × A normalized to lean pups) in *fa/fa* pups. The normalized ROD × A of *fa/fa* rats on P25 was significantly higher than on P2 and 5. The same results were observed in the normalized A, however, the ROD of P25 pups was higher than on P2, 5, and 9. No differences in body weight were observed at any age (Table 1).

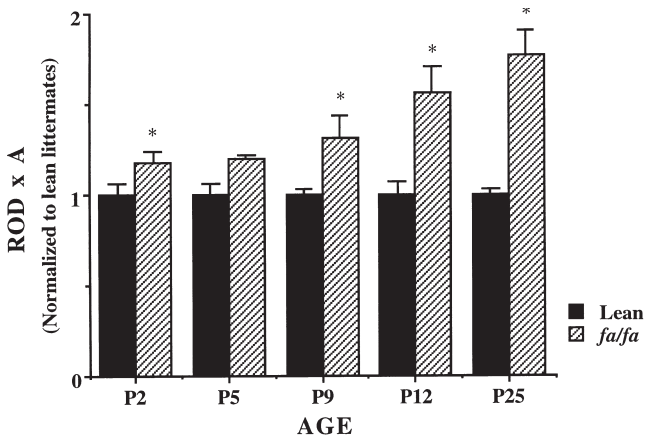

FIG. 1. ROD × A of Zucker rat pups normalized to lean littermates. *Significantly different from lean pups ($p < 0.05$).

The preproNPY mRNA hybridization was observed in small cells within the medial portion of the arcuate nucleus, as previously reported (6). PreproNPY mRNA hybridization was also observed in the cortex, the reticular thalamus, and the hippocampus. Similar levels of preproNPY mRNA hybridization were observed in the cortex and reticular thalamus of *fa/fa* and lean pups on P12 (cortex: 1.00 ± 0.03 versus 0.98 ± 0.12 and reticular n.: 1.00 ± 0.03 versus 0.94 ± 0.10; normalized ROD in lean versus *fa/fa* pups, respectively), indicating that the elevated NPY expression in *fa/fa* animals was specific to the arcuate nucleus.

DISCUSSION

This study shows that the levels of arcuate preproNPY mRNA in *fa/fa* pups are higher than those in lean (+/+ and +/*fa*) pups as early as P2. The presence of preproNPY overexpression on P2, 9, 12, and 25, with a trend towards overexpression on P5, is interpreted as a consistent elevation in arcuate preproNPY mRNA in the *fa/fa* rat from P2. The magnitude of preproNPY overexpression in *fa/fa* rats increases from approximately 1.2-fold higher during the first week of life to a level similar to that previously reported in 5-week-old *fa/fa* rats (29) by P25. Although effects of heterozygozity in Zucker pups have been demonstrated for both fat mass and and fat-free dry mass (33,35), we did not detect differences in preproNPY expression between +/+ and +/*fa* genotypes in this study.

The second postnatal day is the earliest age at which a defect in the expression of a hypothalamic neuropeptide in the *fa/fa* rat has been reported. The onset of preproNPY overexpression in *fa/fa* animals precedes the earliest detection of an

increase in fat mass on P7 (24), and of hyperphagia during independent ingestion on P12 (23). At the time of preproNPY overexpression in *fa/fa* pups at P2, however, decreased energy expenditure is apparent (26). These observations are consistent with the hypothesis that increased hypothalamic NPY activity in the *fa/fa* rat contributes to the decreased energy expenditure and increased food intake with subsequent obesity (36).

Deprivation (4 to 24 h) of rat pups as early as P2 increases the intake of milk or sucrose in ingestive tests independent of the dam (17,18). Additionally, increased NPY expression in response to a 24-h deprivation is observed as early as P2 in lean Zucker rats (22), and exogenous NPY administration to rats aged P2 elicits an increase in water and milk intake in an independent ingestive test (9). These data suggest that shortly after birth, the hypothalamic NPY system is responsive to deprivation, and may be driving independent ingestive behavior as described in the adult. Although *fa/fa* rats overexpress NPY as early as P2, increased suckling has not been observed in the *fa/fa* rat (8,16), while the earliest age that hyperphagia during independent ingestion is observed is P12 (23). Furthermore, others have reported that an increase in hypothalamic NPY content in *fa/fa* rats was observed on P30 but not P16 (3), after hyperphagia is evident. Studies of *fa/fa* pups examining NPY release in the PVN, and the response to exogenous NPY, are required to better define the role of hypothalamic NPY and arcuate preproNPY overexpression in the initiation of hyperphagia.

The increased arcuate preproNPY expression seen with 24 to 72 h of deprivation in adult rats has been attributed to a decrease in circulating leptin associated with deprivation. This is supported by the fact that an absence of leptin or of a functional leptin receptor, as seen in the *ob/ob* mouse and *fa/fa* rat, respectively, results in the overexpression of arcuate preproNPY (30,37). Additionally, central leptin administration decreases arcuate preproNPY expression in deprived rats (32) and in *ob/ob* mice (31). Because *fa/fa* rats overexpress NPY on P2, it is likely that NPY-expressing neurons in the arcuate are sensitive to circulating leptin at this early age. Consistent with this, deprivation of rat pups aged P0 to P1 decreases brown adipose tissue leptin expression and plasma leptin levels (14), while a 24-h deprivation in lean Zucker rats increases arcuate preproNPY expression as early as P2 (22).

In summary, overexpression of arcuate preproNPY is observed in *fa/fa* pups as early as P2. The role of increased preproNPY expression in the emergence of hyperphagia and metabolic disorders in the *fa/fa* rat remains to be determined.

ACKNOWLEDGEMENTS

This work was funded by NIH Grants MH 00149, T32 MH 18390, and MH 15455. We thank Drs. Aron Weller and James Gibbs for constructive criticism of this article.

REFERENCES

1. Allen, Y. S.; Adrian, T. E.; Allen, J. M.; Tatemoto, K.; Crow, T. J.; Bloom, S. R.; Polak, J. M.: Neuropeptide Y distribution in the rat brain. Science 221:877–879; 1983.
2. Bai, F. L.; Yamano, M.; Shiotani, Y.; Emson, P. C.; Smith, A. D.; Powell, J. F.; Tohyama, M.: An arcuato-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Res. 331:172–175; 1985.
3. Beck, B.; Burlet, A.; Bazin, R.; Nicolas, J.-P.; Burlet, C.: Elevated neuropeptide Y in the arcuate nucleus of young obese Zucker rats may contribute to the development of their overeating. J. Nutr. 123:1168–1172; 1993.
4. Beck, B.; Burlet, A.; Nicolas, J.-P.; Burlet, C.: Hypothalamic neuropeptide Y (NPY) in obese Zucker rats: Implications in feeding and sexual behaviors. Physiol. Behav. 47:449–453; 1990.
5. Billington, C. J.; Briggs, J. E.; Grace, M.; Levine, A. S.: Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am. J. Physiol. 260:R321–R327; 1991.
6. Brady, L. S.; Smith, M. A.; Gold, P. W.; Herkenham, M.: Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 52:441–447; 1990.
7. Bray, G. A.; York, D. A.; Fisler, J. S.: Experimental obesity: A homeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. Vitam. Horm. 45:1–125; 1989.
8. Buchberger, P.; Schmidt, I.: Is the onset of obesity in suckling fa/fa rats linked to a potentially larger milk intake? Am. J. Physiol. 271:R472–R476; 1996.
9. Capuano, C. A.; Leibowitz, S. F.; Barr, G. A.: Effect of paraventricular injection of neuropeptide Y on milk and water intake of preweanling rats. Neuropeptide 24:177–182; 1993.
10. Chua, S. C., Jr.; Chung, W. K.; Wu–Peng, X. S.; Zhang, Y.; Liu, S. M.; Tartaglia, L.; Leibel, R. L.: Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271:994–996; 1996.
11. Chua, S. C., Jr.; White, D. W.; Wu–Peng, X. S.; Liu, S. M.; Okada, N.; Kershaw, E. E.; Chung, W. K.; Power–Kehoe, L.; Chua, M.; Tartaglia, L. A.; Leibel, R. L.: Phenotype of fatty due to Gln269Pro mutation in the leptin receptor (Lepr). Diabetes 45:1141–1143; 1996.
12. Chung, W. K.; Truett, G. E.; Smoller, J. W.; Hirsch, J.; Leibel, R.: Increased hypothalamic preproneuropeptide Y mRNA in six to nine day old fatty (fa/fa) rats (abstract). FASEB J. 6:A1785; 1992.
13. Cusin, I.; Rohner–Jeanrenaud, F.; Stricker–Krongrad, A.; Jeanrenaud, B.: The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats. Reduced sensitivity compared with lean animals. Diabetes 45: 1446–1450; 1996.
14. Dessolin, S.; Schalling, M.; Champigny, O.; Lonnqvist, F.; Ailhaud, G.; Dani, C.; Ricquier, D.: Leptin gene is expressed in rat brown adipose tissue at birth. FASEB J. 11:382–387; 1997.
15. Dryden, S.; Pickavance, S.; Frankish, H. M.; Williams, G.: Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats. Brain Res. 690:185–188; 1995.
16. Godbole, V. Y.; Grundleger, M. L.; Pasquine, T. A.; Thenen, S. W.: Composition of rat milk from day 5 to 20 of lactation and milk intake of lean and preobese Zucker pups. J. Nutr. 111:480–487; 1981.
17. Hall, W. G.: The ontogeny of feeding in rats: I. Ingestive and behavioral responses to oral infusions. J. Comp. Physiol. Psychol. 93:977–1000; 1979.
18. Hall, W. G.; Bryan, T. E.: The ontogeny of feeding in rats: II. Independent ingestive behavior. J. Comp. Physiol. Psychol. 94:746–756; 1980.
19. Higuchi, H.; Yang, H. Y.; Sabol, S. L.: Rat neuropeptide Y precursor gene expression. mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester. J. Biol. Chem. 263:6288–6295; 1988.
20. Jhanwar–Uniyal, M.; Chua, S. C.: Critical effects of aging and nutritional state on hypothalamic neuropeptide Y and galanin gene expression in lean and genetically obese Zucker rats. Mol. Brain Res. 19:195–202; 1993.
21. Kalra, S. P.; Dube, M. G.; Sahu, A.; Phelps, C. P.; Kalra, P. S.: Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc. Natl. Acad. Sci. USA 88:10931–10935; 1991.
22. Kowalski, T. J.; Houpt, T. A.; Jahng, J.; Okada, N.; Chua, S. C., Jr.; Smith, G. P.: Ontogeny of neuropeptide Y expression in response to deprivation in lean Zucker rat pups. Am. J. Physiol. 275:R466–R470; 1998.

23. Kowalski, T. J.; Ster, A. M.; Smith, G. P.: Ontogeny of hyperphagia in the Zucker fatty (fa/fa) rat. Am. J. Physiol. 275:R1106–R1109; 1998.
24. Meierfrankenfeld, B.; Abelenda, M.; Jauker, H.; Klingenspor, M.; Kershaw, E. E.; Chua, S. C., Jr.; Leibel, R. L.; Schmidt, I.: Perinatal energy stores and excessive fat deposition in genetically obese (fa/fa) rats. Am. J. Physiol. 270:E700–E708; 1996.
25. Moltz, J. H.; McDonald, J. K.: Neuropeptide Y direct and indirect action on insulin secretion in the rat. Peptides 6:1155–1159; 1985.
26. Moore, B. J.; Armbruster, S. J.; Horwitz, B. A.; Stern, J. S.: Energy expenditure is reduced in preobese 2-day Zucker fa/fa rats. Am. J. Physiol. 249:R262–R265; 1985.
27. Rosenblum, C. I.; Tota, M.; Cully, D.; Smith, T.; Collum, R.; Qureshi, S.; Hess, J. F.; Phillips, M. S.; Hey, P. J.; Vongs, A.; Fong, T. M.; Xu, L.; Chen, H. Y.; Smith, R. G.; Schindler, C.; Van der Ploeg, L. H.: Functional STAT 1 and 3 signaling by the leptin receptor (OB-R); Reduced expression of the rat fatty leptin receptor in transfected cells. Endocrinology 137:5178–5181; 1996.
28. Sahu, A.; Kalra, P. S.; Kalra, S. P.: Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides 9:83–86; 1988.
29. Sancora, G.; Finklestein, J. A.; White, J. D.: Developmental aspects of differences in hypothalamic preproneuropeptide Y messenger ribonucleic acid content in lean and genetically obese Zucker rats. J. Neuroendocrinol. 4:353–357; 1992.
30. Sancora, G.; Kershaw, M.; Finklestein, J. A.; White, J. D.: Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 127:730–737; 1990.
31. Schwartz, M. W.; Baskin, D. G.; Bukowski, T. R.; Kuijper, J. L.; Foster, D.; Lasser, G.; Prunkard, D. E.; Porte, D., Jr.; Woods, S. C.; Seeley, R. J.; Weigle, D. S.: Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes. 45:531–535; 1996.
32. Schwartz, M. W.; Seeley, R. J.; Campfield, L. A.; Burn, P.; Baskin, D. G.: Identification of targets of leptin action in rat hypothalamus. J. Clin. Invest. 98:1101–1106; 1996.
33. Schwarzer, K.; Doring, H.; Schmidt, I.: Different physiological traits underlying increased body fat of fatty (fa/fa) and heterozygous (+/fa) rats. Am. J. Physiol. 272:E100–E106; 1997.
34. Stanley, B. G.; Leibowitz, S. F.: Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 35:2635–2642; 1984.
35. Truett, G. E.; Tempelman, R. J.; Walker, J. A.: Codominant effects of the fatty (fa) gene during early development of obesity. Am. J. Physiol. 268:E15–E20; 1995.
36. White, B. D.; Martin, R. J.: Evidence for a central mechanism of obesity in the Zucker rat: Role of neuropeptide Y and leptin. Proc. Soc. Exp. Biol. Med. 214:222–232; 1997.
37. Wilding, J. P. H.; Gilbey, S. G.; Bailey, C. J.; Batt, R. A. L.; Williams, G.; Ghatei, M. A.; Bloom, S. R.: Increased neuropeptide Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. Endocrinology 132:1939–1944; 1993.
38. Williams, G.; Cardoso, H. M.; Domin, J.; Ghatei, M. A.; Russell, J. C.; Bloom, S. R.: Hypothalamic regulatory peptide disturbances in the spontaneously obese JCR: LA-corpulent rat. Diabetes Res. 15:1–7; 1990.
39. Wu–Peng, X. S.; Chua, S. C.; Okada, N.; Liu, S.-M.; Nicolson, M.; Leibel, R.: Phenotype of the obese Koletsky (*f*) rat due to Tyr763Stop mutation in the extracellular domain of the leptin receptor (Lepr). Evidence for deficient plasma-to-CSF transport of leptin in both the Zucker and Koletsky obese rat. Diabetes 46:513–518; 1997.
40. Yamashita, T.; Murakami, T.; Iida, M.; Kuwajima, M.; Shima, K.: Leptin receptor of Zucker fatty rat performs reduced signal transduction. Diabetes 46:1077–1080; 1997.
41. Zarjevski, N.; Cusin, I.; Vettor, R.; Rohner–Jeanrenaud, F.; Jeanrenaud, B.: Chronic intracerebroventricular neuropeptide Y administration to normal rats mimics hormonal and metabolic changes of obesity. Endocrinology 133:1753–1758; 1993.
42. Zucker, L. M.; Zucker, T. F.: Fatty, a new mutation in the rat. J. Hered. 52:275–278; 1961.