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Induction and reversibility of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats

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Summary Intracerebroventricular neuropeptide Y (NPY) administration to normal rats for 7 days produced a sustained, threefold increase in food intake, resulting in a body weight gain of more than 40 g. Basal plasma insulin and triglyceride levels were increased in NPY-treated compared to vehicle-infused rats by about four- and two-fold, respectively. The glucose utilization index of white adipose tissue, measured by the labelled 2-deoxy-D-glucose technique was four times higher in NPY-treated rats compared to controls. This change was accompanied by an increase in the insulin responsive glucose transporter protein (GLUT 4). In marked contrast, muscle glucose utilization was decreased in NPY-treated compared to vehicle-infused animals. This change was accompanied by an increase in triglyceride content. When NPY-treated rats were prevented from overeating, there was no decrease in muscle glucose uptake, nor was there an increase in muscle triglyceride content. This suggests that muscle insulin resistance of ad libitum-fed NPY-treated rats is due to a glucose-fatty acid (Randle) cycle. When intracerebroventricular NPY administration was stopped and rats kept without any treatment for 7 additional days, all the abnormalities brought about by the neuropeptide were normalized. A tonic central effect of NPY is therefore needed to elicit and maintain most of the hormonal and metabolic abnormalities observed in the present study. Such abnormalities are analogous to those seen in the dynamic phase of obesity syndromes in which high hypothalamic NPY levels have been reported. [Diabetologia (1994) 37: 1202–1208]

Key words Intracerebroventricular (i.c.v.), neuropeptide Y (NPY), food intake, body weight gain, in vivo glucose uptake, muscle insulin resistance.

Obesity is a pathological condition characterized by an imbalance between caloric intake and total energy expenditure. Hyperinsulinaemia and insulin resistance are the most prominent facets of this syndrome. The initial cause(s) that ultimately lead to obesity are yet to be determined. In man, cross-sectional studies suggested the existence of a progressive evolution of the obese subjects to hyperinsulinaemia, subsequent glucose intolerance and diabetes due to pancreatic decompensation. Such studies failed, however, to pin-point any initial cause(s) in such series of events [1, 2].

Investigation carried out in animal models potentially allows for an understanding of the aetiology of obesity syndromes. In this respect, it is noteworthy that chronic intracerebroventricular (i.c.v.) neuropeptide Y (NPY) administration to normal rats produced several of the behavioral, hormonal, and metabolic changes observed in the dynamic phase of the genetic or hypothalamic obesity syndromes [3–8]. Thus, as in young genetically obese animals, chronic i.c.v. NPY administration to normal rats for 7 days resulted in increased food intake, body weight, liver and adipose tissue lipogenic activity, together with a state of muscle insulin resistance [9, 10].

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Abbreviations: NPY, Neuropeptide Y; icv, intracerebroventricular; GLUT 4, glucose transporter 4.

These considerations, together with the reports showing that hypothalamic NPY mRNA and protein expression were both increased during the dynamic phase of these syndromes [11–13] lead to the proposal that NPY could play a role in the establishment and maintenance of obesity syndromes. To further substantiate this viewpoint, NPY was administered i.c.v. to normal rats for 7 days, then the administration of the peptide was stopped and the hormonal-metabolic consequences of such cessation of NPY infusion were determined. To try to distinguish between the effects of NPY per se and those due to NPY-induced hyperphagia, metabolic parameters were determined in NPY-treated rats prevented from overeating (pair-feeding).

Materials and methods

Twelve-week-old lean female rats of the Zucker (FA/?) strain were used throughout the study. The animals were initially purchased from the "Centre de Sélection et d'Elevage d'Animaux de Laboratoire" (Orléans, France). They were bred and housed in our animal quarter, submitted to a 12-h light cycle (lights on from 07.00–19.00 hours) and kept at a constant temperature (23 °C). They were fed a standard laboratory chow (carbohydrate 57.7 %; fat 2.5 %; protein 20.6 %; ash 7.5 %; water 11.7 %, Provimi Lacta, Cossonay, Switzerland). Three days before the implantation of the intracerebroventricular (i.c.v.) guiding cannulas, the rats were placed into individual cages. Body weight and food intake were then measured daily until the end of the experimental period. The i.c.v. cannulation and the subsequent experiments were approved by the Ethical Committee for Animal Experimentation of the Geneva Faculty of Medicine, as well as by the Swiss Federal and Geneva Cantonal Veterinarian Offices.

Surgical procedure and experimental designs: After three days of habituation to their new housing conditions, the animals were anaesthetized with sodium pentobarbital (60 mg/kg) for the placement of guiding cannulas (outer diameter 0.7 mm) into the right lateral cerebral ventricle according to coordinates previously reported [14]. Guiding cannulas were then filled with a stylet and fixed on the skull with dental cement [14]. After a week of recovery, the stylets were removed and replaced by injecting cannulas connected, via a polyethylene catheter, to osmotic minipumps (Model 2001; Alza Corp., Palo Alto, Calif., USA) containing either porcine Neuropeptide Y, NPY (Bachem, Bubendorf, Switzerland), or its vehicle (0.04 mol/l phosphate-buffered saline with 0.1 % bovine serum albumin and 0.01 % ascorbic acid). The minipumps were placed subcutaneously in the interscapular region, as previously described [15].

The animals infused with i.c.v. NPY received 10 μg/day of the peptide for 7 days. One group of control and NPY-treated animals were allowed to eat ad libitum throughout the 7-day experimental period. A second group of i.c.v. NPY-treated animals and their vehicle-infused controls, were, at day 7, submitted to a light ether anaesthesia for removal of minipumps (cessation of NPY administration). Food intake and body weight were again measured daily for 7 additional days. In a third group of control and i.c.v. NPY-treated rats, a pair-feeding experimental design previously described [9] was adopted: animals were allowed to eat ad libitum for the first 3 days. Subsequently, and for the next 4 days of NPY administration, rats were pair-fed to the amount of food eaten by vehicle-treated animals. This was performed to rule out the role of hyperphagia on glucose metabolism together with ensuring successful NPY treatment by the occurrence of an initial hyperphagia (i.e. during the first 3 days). Pair-feeding (four meals per day at identical time intervals) consisted in providing all rats with 85 % of the amount of food consumed by normal rats in a usual ad libitum situation. The aim of this feeding protocol was to increase the avidity of control animals for food, thereby partially mimicking the behaviour of the NPY-treated group.

Euglycaemic-hyperinsulinaemic clamps and measurement of overall glucose metabolism. The first two groups mentioned were tested using the euglycaemic-hyperinsulinaemic clamp technique. They were fasted for 12 h, then anaesthetized with sodium pentobarbital (60 mg/kg) and two indwelling catheters were inserted, one into the right jugular vein for the respective infusion of glucose, human insulin (Actrapid, Novo, Copenhagen, Denmark) and tracers; the other into the left carotid artery for blood sampling, as detailed elsewhere [16]. Body temperature was maintained at 37 °C with a heating blanket connected to a rectal probe. Primed-continuous infusion of U-^{14}C D-glucose (10 μCi/h, New England Nuclear Boston, MA, USA) was initiated to allow for the establishment of a steady state of glucose tracer. Blood samples were taken to determine basal insulinaemia and basal glucose turnover. Insulin was then infused to reach a new steady state of glucose turnover and blood samples were taken for the determination of plasma insulin and glucose specific activity. Hepatic glucose production and rate of total glucose utilization in basal and insulin-stimulated states were calculated as described previously [16].

Determination of insulin-stimulated glucose utilization index in white adipose tissue and in muscles. In the three groups of rats, the glucose utilization index (in vivo glucose uptake) of inguinal white adipose tissue as well as that of eight different muscle types (white and red gastrocnemius, white and red quadriceps, soleus, extensor digitorum longus, tibialis, and diaphragm) was measured during the euglycaemic hyperinsulinaemic clamps associated with the 2-deoxy-D-[^{3}H]-glucose technique as described previously [17, 18].

Analytical procedures relative to clamp studies. Blood samples (50 μl) used for determination of U^{14}C-D-glucose and 2-deoxy-D-[^{3}H]-glucose ([^{3}H]2DG) SPECIFIC ACTIVITIES WERE DEPROTEINIZED WITH 250 μl $ZnSO_4$ (0.3 mol/l) and 250 μl $Ba(OH)_2$ (0.3 mol/l) and immediately centrifuged. The supernatant was used to measure glucose concentration, U^{14}C glucose and [^{3}H]2DG PLASMA RADIOACTIVITY.

For the measurement of basal and insulin-stimulated glucose turnover rate, 200 μl of supernatant was submitted to an ion exchange resin (AG2 × 8; Bio-Rad Laboratories, Richmond, Calif., USA) to avoid concomitant lactate measurement.

Plasma glucose levels were measured by the glucose oxidase method (Glucose analyser 2; Beckman, Palo Alto, Calif., USA). Basal plasma insulin concentrations from the tail tip as well as in samples obtained during the euglycaemic-hyperinsulinaemic clamps (human insulin infusion) were determined in the same assay by a charcoal precipitation radioimmunoassay technique using an antibody directed against both rat and human insulins [19].

Non-esterified fatty acids (NEFA) and triglycerides were measured spectrophotometrically by enzymatic methods using commercial kits (Bio-Mérieux, Marcy l'Etoile, France).

Preparation of total membranes from adipose tissue and measurement of GLUT 4 protein. Inguinal white adipose tissue taken from both i.c.v. NPY or vehicle-administered animals af-

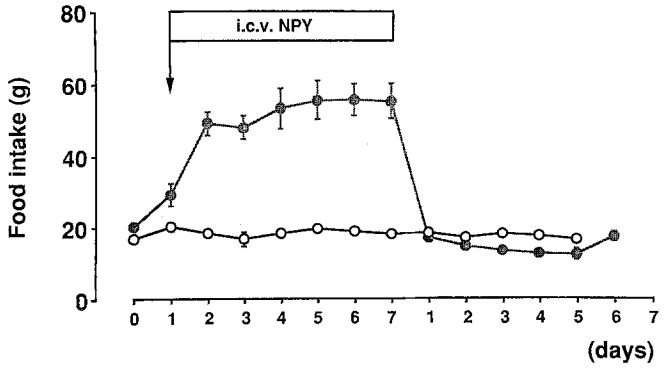

Fig. 1. Daily food intake of normal rats administered i.c.v. NPY for 7 days (●) compared to i.c.v. vehicle-infused controls (○). Cessation of NPY administration at day 7 was achieved by removal of the minipumps delivering the neuropeptide. Means ± SEM of six–eight animals per group. Intergroup differences between day 2 to 7, $p < 0.0001$, between day 1 to 5 (cessation of NPY), NS

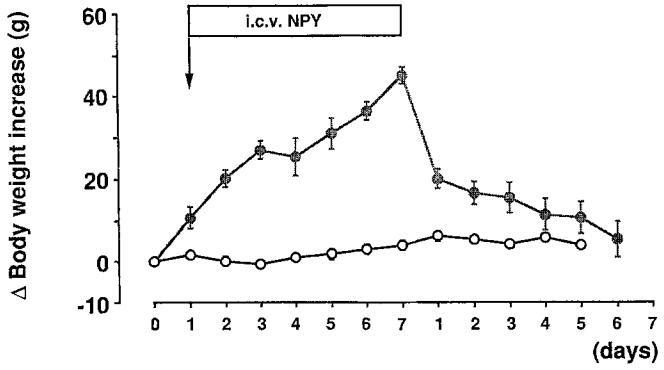

Fig. 2. Delta body weight increase in normal rats administered i.c.v. NPY for 7 days (●) compared to i.c.v. vehicle-infused controls (○). Cessation of NPY administration at day 7 was achieved by removal of the minipumps delivering the neuropeptide. Means ± SEM of six–eight animals per group. Intergroup differences between day 1–2, $p < 0.01$; between day 3–7, $p < 0.0001$; between day 1–3 (cessation of NPY), $p < 0.01$; between day 4–5 (cessation of NPY), NS

ter 7 days of treatment and from animals 7 days after cessation of NPY treatment, was immediately frozen. A total membrane preparation containing both plasma membranes and microsomes was then obtained as previously described [20, 21]. Protein concentration in the resulting membrane preparation was measured as previously reported [22]. Western blots were carried out to measure the relative abundance of the insulin responsive glucose transporter (GLUT 4) protein using a polyclonal antibody against GLUT 4 R820 (Biogenesis LTD, Bournemouth, Dorset, UK) [23–25]. Blots were then submitted to autoradiography. Quantification of GLUT 4 was performed by counting the radioactivity corresponding to the GLUT 4 signal of the autoradiogram, from which background radioactivity was subtracted, from excised pieces of membranes.

Muscle triglyceride assay. One hundred to 200 mg of frozen (−70 °C) tibialis were homogenized in chloroform-methanol (2 : 1 vol : vol). After 4 h of gentle shaking at room temperature, the extract was washed twice with 1 mol/l H_2SO_4 and finally dried in the presence of 100 mg anhydrous $Na_2S_2O_4$. Phospholipids were removed from the samples with preheated silicic acid and evaporated to dryness. Remaining triglycerides were then measured using a standard colorimetric assay [26].

Table 1. Basal biochemical parameters of rats after 7 days of intracerebroventricular (i.c.v.) NPY administration and 7 days after cessation thereof compared to controls

	Plasma glucose (mmol/l)	Plasma insulin (mU/l)	NEFA (mol/l)	Plasma triglyceride (mg/dl)
I.c.v. vehicle-infused	5.1 ± 0.1	42.5 ± 2.5	1.08 ± 0.11	86.4 ± 10.7
I.c.v. NPY	4.7 ± 0.2	197.5 ± 32.5[a]	1.05 ± 0.05	136.7 ± 12.1[a]
Cessation of i.c.v. NPY	5.8 ± 0.4	42.5 ± 2.5	0.99 ± 0.08	65.8 ± 7.0

Samples taken 8 h after food removal. Cessation of i.c.v. NPY administration achieved by removal of minipumps delivering the neuropeptide.
Means ± SEM of 8–11 animals per group. Intergroup differences: [a] $p < 0.001$ vs controls

Statistical analysis

Values are expressed as means ± SEM. Statistical analysis was performed by two tailed Student's *"t"* test for unpaired data. Further analysis by analysis of variance (ANOVA) was performed when indicated.

Results

As shown by Figure 1, intracerebroventricular (i.c.v.) NPY administration to normal rats resulted in an initially rapid, then sustained increase in food intake relative to controls. At day 7, the daily food intake was 55.4 ± 4.9 g in NPY-treated rats and 18.1 ± 0.4 g in vehicle-infused rats ($p < 0.0001$). That such increase in food intake was due to i.c.v. NPY is further shown by Figure 1. Indeed, within 1 day of NPY withdrawal, food intake returned to control values and subsequently remained normal.

Increased food intake resulted, as depicted by Figure 2, in a progressive increase in body weight in the i.c.v. NPY-treated group relative to controls. This was elicited by the presence of i.c.v. NPY as evidenced by the observation that body weight, after cessation of central NPY administration, progressively returned to normal control values (Fig. 2).

As shown by Table 1, prominent increases in basal plasma insulin and triglyceride levels were found in 8-h fasted i.c.v. NPY-treated rats relative to controls, of about four and two fold, respectively. I.c.v. NPY administration did not change plasma glucose and NEFA levels. The increased plasma insulin and triglyceride levels of the i.c.v. NPY-administered animals

Table 2. Parameters of glucose metabolism as assessed by euglycaemic-hyperinsulinaemic clamps in rats after 7 days of i.c.v. NPY treatment and 7 days after cessation thereof compared to controls

	Plasma glucose (mmol/l)	Plasma insulin (mU/l)	Hepatic glucose production ($mg \cdot min^{-1} \cdot kg^{-1}$)	Glucose disappearance ($mg \cdot min^{-1} \cdot kg^{-1}$)	Glucose clearance ($ml \cdot min^{-1} \cdot kg^{-1}$)
Pre clamps					
I.c.v. vehicle-infused	6.9 ± 0.1	58.1 ± 9.9	6.5 ± 0.7	6.5 ± 0.7	5.2 ± 0.5
I.c.v. NPY	6.6 ± 0.1	107.4 ± 21.9[a]	6.6 ± 0.7	6.6 ± 0.7	5.5 ± 0.6
Cessation of i.c.v. NPY	6.6 ± 0.3	77.9 ± 9.3	6.2 ± 0.3	6.2 ± 0.3	5.3 ± 0.9
Post clamps					
I.c.v. vehicle-infused	5.9 ± 0.2	535.0 ± 97.5	0.5 ± 1.0	22.4 ± 1.4	21.1 ± 1.5
I.c.v. NPY	6.6 ± 0.2[a]	672.5 ± 107.5	−1.3 ± 0.4	18.7 ± 0.9[a]	15.7 ± 0.9[a]
Cessation of i.c.v. NPY	6.7 ± 0.3	632.5 ± 65.0	−0.3 ± 0.3	21.7 ± 1.0	18.3 ± 1.2

Animals were fasted for 12 h. Cessation of i.c.v. NPY administration achieved by removal of minipumps delivering the neuropeptide. Means ± SEM of 7–10 animals per group. Intergroup differences: [a] $p < 0.05$ vs controls

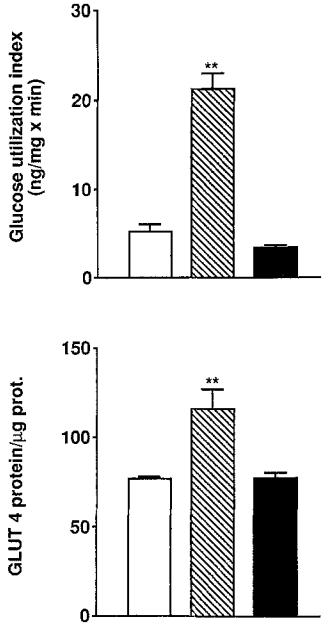

Fig. 3. Inguinal white adipose tissue glucose utilization index (upper panel) and insulin responsive glucose transporter (GLUT 4) protein levels (lower panel) measured in tissues from vehicle-infused normal rats (controls, □); normal rats administered i.c.v. NPY for 7 days (▨); i.c.v. NPY-administered rats for 7 days studied 7 days after cessation of the NPY administration (removal of minipumps delivering the neuropeptide, ■). Glucose utilization index was measured via the labelled 2-deoxyglucose technique during euglycaemic-hyperinsulinaemic clamps (See Materials and Methods). Means ± SEM of six–seven animals per group. Intergroup differences, ** $p < 0.01$

returned to normal values 7 days after cessation of i.c.v. NPY administration.

Clamps were performed in two groups of rats. The first received i.c.v. NPY or its vehicle for 7 days, the other was first administered for 7 days with i.c.v. NPY or its vehicle, then kept for an additional 7-day period following removal of minipumps delivering the neuropeptide. These data are shown in Table 2. It may be seen that all groups were compared at similar steady-state levels of glycaemia and insulinaemia. Insulin inhibited hepatic glucose production normally in all groups. The whole-body glucose disappearance rate was significantly decreased in the group of rats that had been administered with i.c.v. NPY for 7 days relative to respective controls and when expressed per kg body weight while it was unaltered when expressed per animal (data not shown). Rates of glucose disappearance returned to normal values when NPY administration was stopped for 7 days. Similar observations were made for the calculated glucose clearance rates (Table 2). The insulin-stimulated in vivo glucose uptake (or glucose utilization index, as measured by the labelled 2-deoxy-D-glucose technique) by adipose tissue is shown in Figure 3. It may be seen that the inguinal white adipose tissue from 7-day i.c.v. NPY-administered rats was much more insulin responsive than that of vehicle-infused controls. This was accompanied by an increase in the total amount of the insulin responsive glucose transporter (GLUT 4) protein (Fig. 3). That these changes were produced by the presence of NPY within the brain was shown by the observation of a return to control values of both the in vivo adipose tissue glucose utilization index and adipose tissue GLUT 4 protein levels 7 days after cessation of NPY administration (Fig. 3).

As shown in Table 3 glucose utilization index by total interscapular brown adipose tissue was unchanged in i.c.v. NPY-administered rats as compared to controls. After cessation of NPY treatment glucose uptake tended to be lower than that of controls without reaching statistical significance.

The insulin-stimulated in vivo glucose utilization index by several different muscle types is shown by Figure 4. Relative to the glucose utilization index observed in muscles from vehicle-infused rats, the insulin responsiveness of muscles obtained from i.c.v. NPY-treated rats was significantly and often markedly decreased. The decreased insulin responsiveness of muscles from i.c.v. NPY-administered animals was normalized after 7 days of cessation of NPY adminis-

Table 3. Glucose utilization index of total interscapular brown adipose tissue in rats after 7 days of i.c.v. NPY treatment and 7 days after cessation thereof compared to controls

	Glucose utilization index (ng/tissue × min)
I.c.v. vehicle-infused	50.4 ± 9.8
I.c.v. NPY	56.4 ± 9.5
Cessation of i.c.v. NPY	30.2 ± 3.1[a]

Glucose utilization index was measured via the labelled 2-deoxyglucose technique during euglycaemic-hyperinsulinaemic clamps (See Materials and Methods).
Means ± SEM of 6–7 animals per group. Intergroup differences: NS vs vehicle-infused; [a] $p < 0.05$ i.c.v. NPY vs cessation of i.c.v. NPY

Table 4. Glucose utilization index in eight different muscle types in pair-fed i.c.v. NPY-treated rats compared to respective controls

	Glucose utilization index (ng/mg × min)	
	I.c.v. vehicle-infused	I.c.v. NPY
Soleus	25.5 ± 3.0	31.3 ± 3.5
Extensor digitorum longus	23.7 ± 2.6	20.9 ± 1.9
Tibialis	22.0 ± 2.9	24.9 ± 1.5
White gastrocnemius	12.4 ± 1.2	9.9 ± 0.9
Red gastrocnemius	31.3 ± 3.3	24.2 ± 1.7
White quadriceps	17.9 ± 2.4	19.8 ± 1.4
Red quadriceps	42.3 ± 3.8	34.1 ± 1.8
Diaphragm	58.9 ± 2.4	63.0 ± 5.2

Glucose utilization index was measured via the labelled 2-deoxyglucose technique during euglycaemic-hyperinsulinaemic clamps (See Materials and Methods).
Means ± SEM of 6–7 animals per group. Intergroup differences = NS

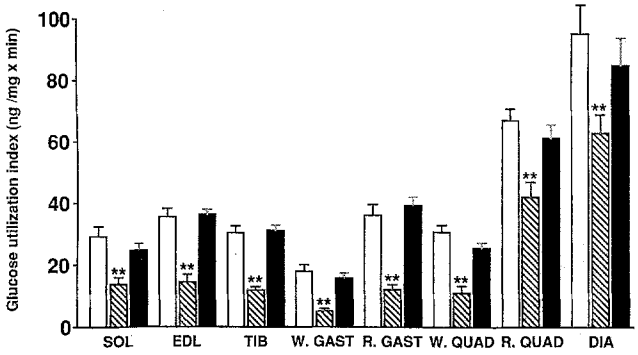

Fig. 4. Muscle glucose utilization index measured in eight different muscle types obtained, respectively, from vehicle-infused normal rats (controls, □); normal rats administered i.c.v. NPY for 7 days (▨); i.c.v. NPY-administered rats for 7 days studied 7 days after cessation of i.c.v. NPY administration (removal of minipumps delivering the neuropeptide, ■). Glucose utilization index was measured via the labelled 2-deoxyglucose technique during euglycaemic-hyperinsulinaemic clamps (See Materials and Methods). SOL, soleus; EDL, extensor digitorum longus; TIB, tibialis; W. GAST, R. GAST, white or red gastrocnemius; W. QUAD, R. QUAD, white or red quadriceps; DIA, diaphragm. Means ± SEM of six–seven animals per group. Intergroup differences indicated by ** $p < 0.01$. Other intergroup values, NS

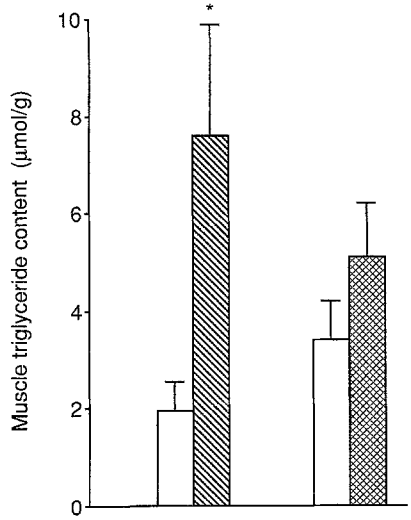

Fig. 5. Muscle triglyceride content measured in tibialis of vehicle-infused normal rats (controls, □); normal rats administered i.c.v. NPY for 7 days fed ad libitum (▨, left panel); i.c.v. NPY-administered rats fed ad libitum for 3 days and subsequently pair-fed to controls for 4 days (▩, right panel). Means ± SEM of six–seven animals per group. Intergroup differences indicated by * $p < 0.05$. Other intergroup values, NS

tration (Fig. 4). The changes observed in muscles from i.c.v. NPY-treated rats were not accompanied by any alteration in the amount of GLUT 4 protein (data not shown). However, there was a marked increase in triglyceride content in insulin-resistant muscles of NPY-treated rats as measured in the tibialis (Fig. 5). Such was not the case in muscles of i.c.v. NPY-treated rats pair-fed to controls for the last 4 days of treatment in which triglyceride content was similar to that of controls (Fig. 5). This is in keeping with the observation of unaltered glucose utilization in muscles of pair-fed i.c.v. NPY-treated rats as compared to respective controls (Table 4).

Discussion

The present study extends previous observations showing that i.c.v. NPY administration to normal rats for 7 days resulted in hyperphagia, increased body weight gain, hyperinsulinaemia, increased white adipose tissue metabolic activity concomitant with insulin resistance at the level of the muscle mass [4, 9, 10]. The hormonal and metabolic alterations observed after i.c.v. NPY administration are interestingly similar to those observed during the dynamic phase of hypothalamic or genetic obesity syndromes [3, 5–8]. As genetically obese rodents are characterized by early overexpression of their hypo-

thalamic NPY protein and mRNA levels [11–13], it was of additional interest to investigate whether cessation of i.c.v. NPY administration would result in the reversal of the metabolic defects brought about by the peptide. If such was the case, it would suggest that attempts at altering NPY levels in young genetically obese rodents (via central NPY antagonist, NPY antibody, or antisense NPY oligonucleotide administration) could possibly prevent the development of their syndrome. Indeed, down-regulation of food intake and of basal insulin levels have already been shown when antisense NPY oligonucleotides were administered directly into the arcuate nucleus of normal rats [27].

The present study shows that 7 days after the cessation of i.c.v. NPY administration to normal rats, increased food intake and body weight gain, hyperinsulinaemia and hypertriglyceridaemia as well as the insulin over-responsiveness of white adipose tissue and the insulin under-responsiveness of all muscles studied had all returned to normal values. Such was also the case for the total glucose disappearance rate that was slightly lower in i.c.v. NPY-treated rats than in controls when expressed per kg body weight (but not when expressed per animal).

For white adipose tissue from i.c.v. NPY-infused rats the sequence of events proposed could be: increase in basal and substrate-induced insulinaemia, over-expression of white adipose tissue GLUT 4 mRNA and protein with resultant increase in glucose transport activity in this tissue. This sequence is in keeping with data obtained upon administering normal rats with insulin for 4 days while maintaining euglycaemia by superimposed glucose infusion [28, 29]. This view is also strengthened by the observation of a normalization of basal insulinaemia, as well as of white adipose tissue GLUT 4 protein and glucose transport, 7 days after cessation of i.c.v. NPY administration.

The mechanism of the effect of i.c.v. NPY on the establishment of muscle insulin resistance of the glucose transport process is less clear, as muscle GLUT 4 expression is unaltered by i.c.v. NPY administration. It could conceivably be due to an increased glucose-fatty acid cycle [30]. Indeed, in an overweight condition, excess body fat is not limited to adipose tissue but is also present in muscles [31]. Additionally, muscle lipid substrate is supplied by hydrolysis of triglycerides inside the muscle fibers [32]. Several observations have thus shown that a large proportion of the increase in lipid oxidation of obese subjects is accounted for by an increase in intramuscular triglyceride mobilization [31, 33–36]. Finally, muscle insulin resistance has been shown to be directly related to the accumulation of muscle triglycerides [26, 37]. This view is substantiated, in the present study, by the observation that in i.c.v. NPY-treated rats prevented from overeating (i.e. pair-feeding experiments), there was no significant increase in muscle triglyceride content (judged by that of tibialis), in contrast to what was observed in muscles of ad libitum-fed NPY-treated rats (Fig. 5). When no triglyceride accumulation was noted in pair-fed NPY-administered rats (presumably due to lesser substrate availability), there was no defect in muscle glucose utilization index (Table 4), while all muscles of hyperphagic NPY-treated animals that had a high triglyceride content were resistant to the in vivo effect of insulin on glucose uptake (Fig. 4). This difference is consistent with the hypothesis that the degree of hyperinsulinaemia (meal-induced insulin responses, in particular) was probably unequal in the two groups of i.c.v. NPY-treated rats. It was probably much more marked in hyperphagic than in pair-fed NPY-treated rats, bringing about muscle insulin resistance by yet another mechanism; i.e., hyperinsulinaemia, also known to produce muscle insulin resistance [28, 29, 38].

I.c.v. NPY treatment of normal rats produced no change in total interscapular brown adipose tissue glucose uptake. This was the result of both an increase in tissue weight and a decrease in glucose utilization as expressed per milligram of tissue in i.c.v. NPY-treated animals compared to controls. Seven days after cessation of NPY treatment, tissue weight had decreased while glucose utilization index expressed per milligram of tissue had remained unaltered compared to NPY-treated rats. Under these conditions, total brown adipose tissue glucose uptake was decreased, although such decrease failed to reach statistical significance compared to vehicle-infused rats.

To conclude, when i.c.v. NPY-administered rats are fed ad libitum and therefore overeat, the hormonal and metabolic homeostasis is altered, with high body weight gain and concomitant muscle insulin resistance presumably due to an increased glucose-fatty acid (Randle) cycle. These defects are normalized 7 days after cessation of i.c.v. NPY treatment. This suggests that a normalization of the high central NPYergic tone of obese rodents in general could result in an amelioration of their obesity-insulin resistance syndrome.

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