Chronic Intracerebroventricular Neuropeptide-Y Administration to Normal Rats Mimics Hormonal and Metabolic Changes of Obesity*

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ABSTRACT

Chronic intracerebroventricular (icv) administration of neuropeptide-Y (NPY; 10 μg/day) was performed in normal female rats to investigate its hormonal and metabolic consequences. Intracerebroventricular NPY produced hyperphagia, increased basal insulinemia, as well as liver and adipose tissue lipogenic activity. It also increased basal morning corticosteronemia. When NPY-induced hyperphagia was prevented by pair-feeding, the icv NPY treatment resulted in the same increases in basal insulinemia and corticosteronemia, and liver and white adipose tissue lipogenesis was still higher than that in respective controls. Under the ad libitum and pair-feeding conditions, icv NPY stimulated glucose uptake as well as total lipoprotein lipase activity in white adipose tissue; it resulted in an increased total activity of hepatic and white adipose tissue acylcoenzyme-A-carboxylase. As all hormonal and metabolic changes elicited by icv NPY remained present (at the same or to a lesser extent depending upon the parameter considered) when hyperphagia was prevented by pair-feeding, it was, thus, shown that icv NPY per se induces peripheral hormonal and metabolic alterations via different routes, which remain to be determined. The effects of icv NPY reported in this study are similar to the defects observed in the early phase of genetic obesity in rodents, the hypothalamus of which has increased NPY levels. NPY could, thus, be of relevance in the occurrence of genetically induced obesity. (Endocrinology 133: 1753–1758, 1993)

NEUROPEPTIDE-Y (NPY) is a 36-amino acid peptide initially isolated from pig brain (1, 2), which has been subsequently found in several species, including rats and other rodents (3). It is named Y for the single letter code identifying the tyrosine residues found at the C- and N-terminals as well as at three other positions in the molecule (3). Most of the metabolic effects of NPY appear to be mediated via the hypothalamic area (3–5). Within this region, NPY is synthesized in the arcuate nucleus (ARC), which produces a dense projection of NPY-containing axons ending mainly in the paraventricular nucleus (PVN) (6).

NPY administered intracerebroventricularly (icv) has been shown to be a potent stimulator of food intake in normal rats (7–10). It produces obesity when its administration is maintained for several days (11, 12). Acute central NPY administration to normal rats has been shown to increase plasma insulin levels (13, 14), stimulate white adipose tissue lipoprotein lipase activity, and inhibit the thermogenic capacity of brown adipose tissue (15). Other studies have shown that hypothalamic NPY or NPY mRNA content is increased in genetically obese rodents (e.g. the fa/fa rat and the cp/cp rat) (16–23), animals in which food intake, plasma insulin levels, and hepatic and adipose tissue lipogenic activities have been shown to be increased while energy dissipation is decreased (24).

Due to these considerations, the aim of the present study was to investigate whether chronic (7-day) icv NPY administration to normal rats would result in hormonal and metabolic defects similar to those in genetically obese rats. The impact of NPY in the absence of hyperphagia (pair-feeding experiments) on hormone output as well as on glucose and lipid handling by liver and adipose tissue was also investigated to determine the NPY-induced changes that are not linked to hyperphagia.

Materials and Methods

Animals

Twelve-week-old lean female rats of the Zucker (FA/FA) strain were used throughout the study. The animals were initially purchased from the Centre de Sélection et d'Elevage d'Animala de Laboratoire (Orleans, France). They were bred and housed in our animal quarters, submitted to a 12-h high light cycle (lights on from 0700–1900 h), kept at a constant temperature (23 C), and fed a standard laboratory chow (Provimi Lacta, Cossonay, Switzerland). Three days before implantation of the icv guiding cannulas, the rats were placed into individual cages. Body weight and food intake were then measured daily until the end of the experiments. The experiments were approved by the Geneva Committee on Animal Experimentation.

Surgical procedure and experimental designs

After 3 days of habituation to their new housing conditions, the animals were anesthetized with sodium pentobarbital (60 mg/kg) for the placement of guiding cannulas (od. 0.7 mm) into the right lateral cerebral ventricle according to coordinates previously reported (25). Guiding cannulas were then filled with a styliet and fixed on the skull with dental cement (25). After a week of recovery, the styliets were removed and replaced by injecting cannulas connected, via a polyethylene catheter, to osmotic minipumps (model 2001, Alza Corp., Palo Alto, CA) containing either porcine NPY (Bachem, Bubendorf, Switzerland) or its vehicle (0.04 μ PBS with 0.1% BSA and 0.01% ascorbic acid).
In all of the experimental groups described below, the animals treated with icv NPY received 10 μg/day of the peptide for 7 days. This icv NPY dose was chosen because it was just above the minimally effective one that reportedly stimulates food intake and body weight gain (27). Morning blood samples were always collected from the tip of the tail before the beginning and at the end of the 7-day experimental period for determinations of hormones and metabolites.

In experimental group A, control and NPY-treated animals were allowed to eat ad libitum throughout the 7 days of the experiment. In experimental group B, the animals were allowed to eat ad libitum for the first 3 days after the beginning of the treatment. Subsequently, NPY-treated rats were pair-fed with vehicle-treated control animals for the next 4 days of NPY administration. This enabled us to make the diagnosis of successful NPY administration by the observation of an initial increase in food intake and to rule out a role of hyperphagia, using pair-feeding, on the results obtained. Pair-feeding consisted of providing all rats with 85% of the amount of food consumed by control animals in the ad libitum situation; this amount was divided into four meals per day, with the following schedule: 000, 700, 1200, and 1800 h. The decrease in the amount of food given to controls was adopted to increase their avidity for food, thereby partially mimicking the avidity for food of the NPY-treated group and resulting in a similar timing of diagnosis of successful NPY administration by the observation of an increased food intake (controls (n = 8), 20.0 ± 0.5 g/day; NPY-treated, 34.0 ± 1.9 g (n = 12); intergroup difference on day 3, P < 0.001]. This permitted the precise diagnosis of adequate NPY treatment.

To further rule out the role of hyperphagia even when transient, an additional series of experiments was carried out (group C). In experimental group C, pair-feeding identical to that just described was used, except that it began on day 1, i.e. at the start of icv NPY administration, and lasted for the 7-day experimental period. Successful NPY treatment of group C was assessed by an increase in food seeking and adequacy of placement of the cannulae into the lateral cerebral ventricle, using trypsin blue injection at the end of the experiments. Based on these criteria, i.e. lack of food seeking and absence of trypsin blue in the ventricular system, only one of nine NPY-treated animals was rejected.

Determination of de novo lipogenesis

On the last day of the experiment in groups A, B, and C, control and NPY-treated animals were injected ip with 2 mCi 3H2O (New England Nuclear, Boston, MA); 2 h after food removal for group A or 2 h after the last meal for groups B and C. Blood was collected from the tip of the tail at 30 and 50 min for determination of 3H2O specific activity. At 30 min, rats were killed by decapitation, and liver and inguinal white adipose tissue were removed and frozen. Lipids were extracted (28) and saponified (29) to measure the rate of incorporation of 3H into fatty acids, which was expressed, taking a plasma water content of 55% (28) and a molecular weight of 34, m, as microatoms per h and per g tissue.

Determination of total acetyl coenzyme-A carboxylase (ACC) and lipoprotein lipase (LPL) activities

At the end of NPY administration to another series of animals (protocols A and B), both white adipose tissue and liver were removed to measure total ACC activity. Tissues (inguinal white adipose tissue and liver) were homogenized in a 100 mM KH2PO4 buffer containing 2 mM EDTA, pH 7.3. After a 30-min centrifugation at 10,000 rpm, the supernatants were passed through a Sephadex G-25 column to remove small molecular species. Total ACC activity was then measured as described previously (30), except NADH and lactate dehydrogenase were added to the assay medium to avoid concomitant pyruvate carboxylase measurement. Total ACC activity was expressed as nanomoles of H14CO3− (New England Nuclear, Boston, MA) incorporation into malonyl coenzyme-A per min and per mg protein.

Using protocols A and B, inguinal white adipose tissue from control and NPY-treated rats was also used for the measurement of its total LPL activity. Adipose tissue (~250 mg) was homogenized in 2 ml 10 mM Tris, 0.15 M NaCl, 1 μg/ml aprotinin, and 1 μg/ml pepstatin, pH 7.4, to which 500 μl 1% Triton X-114 were added. The homogenate was then solubilized for 5 min at 20°C and for 30 min at 4°C to unmask all latent LPL activity. The homogenate was incubated in closed membrane vesicles (31). Triton X-114 was allowed to precipitate for 8 min at 30°C, and the supernatant was used to measure total LPL activity. LPL activity was determined by the serum-dependent hydrolysis of [9,10-3H]triolein (New England Nuclear), as described previously (31). LPL activity was expressed as microunits per mg protein (i.e. picomoles of fatty acid produced per min/mg protein).

Determination of insulin-stimulated glucose utilization index in white adipose tissue

The glucose utilization index of inguinal white adipose tissue of control and NPY-treated rats (protocols A and B) was measured in additional groups of animals during euglycemic hyperinsulinemic clamps associated with the 2-deoxy-o-[1-3H]glucose technique (32). Clamps were performed on day 7 after an overnight fast. Rats were anesthetized with sodium pentobarbital (60 mg/kg); catheters were inserted into the right jugular vein for infusion of glucose, insulin, and 2-deoxy-o-[1-3H]glucose (30 μCi; Amersham, Aylesbury, Buckinghamshire, United Kingdom) and into the left carotid artery for blood sampling. Measurements of blood glucose and tracer specific radioactivities were performed as detailed previously (33). After removal of inguinal white adipose tissue, the content of 2-deoxy-o-[1-3H]glucose-6-phosphate was determined to measure the glucose utilization index, expressed as nanograms per min/mg protein, as previously described (34).

Hormonal and metabolic measurements

Plasma glucose levels were determined by the glucose oxidase method (Glucose Analyzer 2, Beckman, Palo Alto, CA). Plasma insulin (35) and corticosterone (36) levels were measured by RIA. Plasma triglyceride levels were determined using kits (Bio-Merieux, Marcy l’Etoile, France). Tissue protein content was measured using the Bio-Rad Assay Kit (Munich, Germany), with BSA as standard.

Statistical analysis

Statistics were performed using two-tailed Student's t test for paired (insulin results) or unpaired data (other results). For some parameters, further statistical analysis of the data by analysis of variance was also carried out.

Results

Rats treated with NPY and fed ad libitum for 7 days were significantly heavier at the end of the treatment than control rats fed ad libitum (group A). NPY-treated animals submitted to pair-feeding (groups B and C) did not show significant differences in body weight compared to control rats. However, the inguinal white adipose tissue weight was greater in NPY-treated rats compared to controls regardless of the feeding paradigm used. Blood glucose levels were slightly decreased in NPY-treated animals of groups A and B compared to those in the respective controls. Plasma triglyceride levels were increased in all NPY-treated groups relative to those in the respective controls (Table 1).

As shown in Fig. 1, basal plasma insulin levels (i.e. measured 2 h after the last meal) were markedly elevated in all NPY-treated groups (A, B, and C), i.e. whatever the feeding conditions used. The icv NPY administration also resulted in increases in liver and white adipose tissue de novo lipogenesis (Fig. 2). For these latter parameters, the increases were highest in the NPY-treated group with the greatest availability of
TABLE 1. Basal characteristics of NPY-treated rats and respective controls

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>BW (g)</th>
<th>Inguinal white adipose tissue weight (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (fed ad libitum)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>204 ± 2</td>
<td>1.4 ± 0.1</td>
<td>129 ± 2</td>
</tr>
<tr>
<td>NPY-treated</td>
<td>5</td>
<td>225 ± 8*</td>
<td>2.2 ± 0.3*</td>
<td>108 ± 2*</td>
</tr>
<tr>
<td><strong>Group B (3-day ad libitum feeding, 4-day food restriction)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>198 ± 3</td>
<td>1.1 ± 0.2</td>
<td>115 ± 1</td>
</tr>
<tr>
<td>NPY-treated</td>
<td>7</td>
<td>197 ± 3</td>
<td>2.1 ± 0.2*</td>
<td>105 ± 2*</td>
</tr>
<tr>
<td><strong>Group C (7-day food restriction)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>192 ± 6*</td>
<td>0.9 ± 0.1</td>
<td>101 ± 1</td>
</tr>
<tr>
<td>NPY-treated</td>
<td>8</td>
<td>181 ± 3*</td>
<td>1.4 ± 0.1*</td>
<td>99 ± 1</td>
</tr>
</tbody>
</table>

For definition of respective groups, see Materials and Methods. Body weights refer to the final body weight, i.e. at the end of the 7-day experimental period. Values are the mean ± SEM of n experiments, as indicated. P = NS unless otherwise indicated. The mean body weight of the animals before the experiment was 191 ± 2 g (n = 33). The body weight change during the 7-day NPY treatment was: Group A, +32.6 ± 4.9 g (P < 0.003); group B, +1.9 ± 1.4 g (P = NS); group C, −7.1 ± 2.5 g (P < 0.05).

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Discussion

NPY has been, since its discovery in 1982 (1, 2), the subject of many investigations, which have emphasized its importance in stimulating food intake and decreasing energy expenditure, with resulting obesity (3-5).

The present study is the first to determine the effects of chronic (7-day) icv NPY administration to normal rats on some hormone levels and several facets of glucose and lipid metabolism. It is also the first to compare the chronic hormonal-metabolic effects of icv NPY in hyperphagic as well as nonhyperphagic animals, thereby enabling the detection of genuine NPY effects, i.e. not related to hyperphagia.

This work shows that chronic icv NPY administration to ad libitum-fed normal rats resulted in marked hyperphagia and increased body weight, in keeping with analogous studies carried out by others (7-12). In addition, chronic icv NPY treatment produced elevated morning plasma corticosterone levels whether the animals were fed ad libitum or pair-fed. This NPY effect may be mediated via a stimulation of CRF, as has been suggested by others using acute experiments (37, 38).

When measuring basal insulinemia, it was observed that the latter was increased to a similar extent regardless of whether the NPY-treated rats were fed ad libitum or pair-fed to the amount of food given to control animals either for 4 days (group B) or for the entire experimental time (group C).

Such results are worth emphasizing, as they suggest that NPY per se, in the absence of hyperphagia, increases peripheral plasma insulin levels, possibly via the parasympathetic nervous system, as has been proposed, albeit via acute experiments (14). Moreover, several studies have suggested the regulation of hypothalamic NPY by insulin (39-45). It has been observed that low plasma insulin levels (e.g. food-
restricted normal rats) are accompanied by increased NPY or NPY mRNA levels at the level of the PVN and ARC in particular (39–43). When plasma insulin levels increase upon refeeding (39) or when insulin is administered icv to normal rats, central NPY mRNA levels decrease (44, 45). These data and those of the present study further support the existence of functional relationships between NPY and insulin, whereby central NPY stimulates insulin release, whereas insulin down-regulates central NPY levels in normal rats. Such regulation would be abnormal in genetically obese rats, in which insulin fails to down-regulate central NPY levels, which, therefore, remain elevated (44).

In this work, it was further shown that whatever the feeding conditions used, icv NPY administration stimulated the overall de novo lipogenesis in liver and adipose tissue (measured by \(^{3}H\) incorporation into long chain fatty acids). The extent of these increases in de novo lipogenesis was highest, however, in those animals in which substrate availability was the greatest, i.e. in the ad libitum-fed NPY-treated animals.

The observed increase in overall de novo lipogenesis observed in NPY-treated groups (i.e. ad libitum-fed or pair-fed animals) was the result of at least the three combined mechanisms measured: 1) an increase (of analogous magnitude whether the animals were fed ad libitum or pair-fed) in the in vivo glucose uptake by white adipose tissue together with increased channelling of glucose carbons into long chain fatty acids, as evidenced by the increased total activity of the adipose tissue ACC, 2) an increase in the uptake of circulating triglycerides demonstrated by the increase in the total activity of white adipose tissue LPL, 3) an increased hepatic de novo lipogenesis in keeping with the observed increase in hepatic ACC activity.

It has been previously reported that the genetically obese fa/fa rat as well as other genetic models of obesity in rodents are characterized by hyperphagia, hyperinsulinemia (24, 46), increased liver and adipose tissue lipogenic activities (47), varying degree of insulin resistance (24, 33), and hypercorticism (48, 49) together with decreased energy expenditure (50). As mentioned above, increased NPY levels have been measured in discrete hypothalamic nuclei of genetically obese rodents, in the ARC and PVN in particular (3, 16–23). It is, therefore, of interest to note that many of the hormonal and metabolic defects of the genetically obese rodents as well as obese humans, including hyperinsulinemia, hypercorticism, and increased fat accretion (48, 48, 51), have been mimicked in the present study by the chronic icv administration of NPY to ad libitum-fed or pair-fed normal rats.

It is even more important to realize that the hormonal and metabolic changes brought about by icv NPY can be observed in the absence of hyperphagia. This indicates the existence of genuine NPY effects arising from the brain and modifying peripheral homeostasis. The routes suggested above for these NPY-induced peripheral changes (concomitant increases in the hypothalano-pituitary-adrenal axis and efferent parasympathetic activities), actually remain to be demonstrated. According to the considerations just mentioned, NPY-induced hyperphagia would, by increasing substrate availability, be only an aggravating factor, rather than a necessary one, in establishment of the alterations described in this study. Such may also be the case in genetically obese rodents, in which the NPYergic system in the hypothalamus is reportedly overactive and could be responsible for several features of their syndrome (52).

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