Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism

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Billington, C. J., J. E. Briggs, M. Grace, and A. S. Levine. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. Am. J. Physiol. 260 (Regulatory Integrative Comp. Physiol. 29): R321-R327, 1991.—Our objective was to find out if central injection of neuropeptide Y (NPY) would alter brown fat thermogenesis and white fat lipoprotein lipase activity. The following three groups of Sprague-Dawley rats received five injections over 24 h into the right lateral ventricle: 1) NPY (5 μg/injection) and ad libitum food; 2) NPY (5 μg/injection) and food restricted to control intake; 3) saline injection and ad libitum food. The NPY ad libitum-fed group consumed more food than the saline controls or NPY food-restricted animals. Brown fat thermogenic activity, assessed by GDP binding, was decreased relative to saline controls in both NPY-treated groups. White fat lipoprotein lipase activity was greatly increased in both NPY treatment groups compared with saline controls. The NPY effects on brown and white fat were not explained by measures of serum insulin, glucagon, glucose, or other metabolites. In a follow-up experiment, we asked whether food was necessary for expression of the NPY effects. Brown fat mitochondrial GDP binding indicated NPY effect even when no food was ingested. We conclude that intracerebroventricular administration of NPY promotes white fat lipid storage and decreases brown fat thermogenesis in addition to its known effect of stimulating food intake.

METHODS

The experimental design and procedures were approved by the Institutional Animal Studies Committee and were conducted in accord with the Helsinki declarations. For these experiments, the animals were male Sprague-Dawley rats of weight 200–350 g. The animals were housed individually in steel-mesh cages with ad libitum access to rat chow and water, except as otherwise noted. The animals were maintained in the same room in the animal colony and experienced a 12:12 light-dark cycle. Mean room temperature during these studies was 21°C.

Stereotaxic procedures for implanting cannulas. For each of the experiments, the animals were prepared with a right ventricular cannula. The animals were anesthetized for this procedure. The stereotaxic coordinates were those of Paxinos and Watson (28). The stereotaxic instrument (David Kopf) was used to locate specific brain structures in three planes with respect to skull landmarks (bregma, ear-bar zero). An incision was made and a small hole was drilled through which the cannula was lowered. At least four additional short holes were drilled, into which self-tapping stainless steel screws were placed. Once the cannula was lowered to the appropriate coordinates, dental acrylic cement was applied to anchor the

brown adipose tissue; lipoprotein lipase; adipose tissue

Perhaps the most intriguing known effect of the neuropeptide Y (NPY) is potent stimulation of feeding. The effect of NPY on feeding was first noted by Clark and colleagues (8) and has been studied by Stanley and Leibowitz (34, 35) and by our group (20, 25) as well as many others. This feeding effect is seen after intracerebroventricular (icv) administration of NPY, as well as at specific brain sites, notably the paraventricular nucleus of the hypothalamus (34, 35). The effect of NPY is extremely potent, probably the most powerful stimulus currently known for provoking feeding. In addition, it has been observed that NPY can provoke food intake in a sated animal (20, 27) and that this effect of NPY can be seen with each repetitive injection when given every few hours (25). Consequently, prodigious quantities of food consumption can be stimulated in rats and in other animals in response to repetitive stimulation by NPY.

Since the observations of Rothwell and Stock (29), it has been known that animals induced to overeat by provision of a palatable “cafeteria” diet show induction of a thermogenic mechanism, which in rats is primarily expressed in brown adipose tissue. Similar observations of induced thermogenesis in rat brown fat have been seen in response to overfeeding induced with sucrose solutions (36). In addition, a large body of literature has chronicled investigations into diet-induced thermogenesis in a single meal setting in humans and other larger animals (e.g., Refs. 31, 32).

Recognizing that NPY stimulates feeding and that feeding has been shown to stimulate brown fat thermogenesis, we hypothesized that NPY might also stimulate brown fat thermogenesis when it was used to produce overfeeding in rats. Consequently, we designed an experiment to test this hypothesis, including a group treated with NPY but not allowed to overeat as a control for the effects of NPY itself. The results of that experiment were not in accord with our original hypothesis. We found that NPY decreased brown adipose tissue thermogenesis, an effect that was not a consequence of the increase in food intake it produced.
cannula to the screws. Cannulas were closed with a stainless steel stylet when not in use. Animals were allowed to recover for a minimum of 5 days before beginning any experiment.

Injection procedure. Injection cannulas were fabricated from 30-gauge stainless steel tubing, which are cut such that when inserted to maximum depth they protrude 0.5 mm beyond the tips of the guide cannulas. These cannulas were attached by polyethylene tubing to microsyringes (Hamilton, 10 μl). Injection volume was 0.5 μl for lateral ventricular injections given slowly over 30 s. Cannulas were left in place an additional 15 s to allow diffusion from the tip. After injection, the cannula was withdrawn, the stylet replaced, and the rat returned to its home cage.

Verification of cannulas. After death, the icv cannulas were injected with India ink, and placement in the ventricular system was verified by direct observation of ventricular fluid or the ventricular system itself. Data from rats with incorrect placement were discarded.

Experiment 1. Three groups of eight rats were prepared with cannulas. Each group received five icv injections spaced every 6 h and beginning at 8:00 A.M. The final injection was at 8:00 A.M. on the following day. The animals were rapidly killed 2 h after the last injection, and samples were obtained of serum for glucose, glucagon, insulin, lactate, pyruvate, hydroxybutyrate, acetoacetate, and free fatty acids. In addition, the interscapular brown fat was dissected free and processed, as indicated below, for GDP binding, mitochondrial protein content, and cytochrome oxidase activity. Perirenal white fat was also obtained and later assayed for lipoprotein lipase activity. The three groups each received a different treatment according to the following. 1) The saline group received icv injections of saline in a volume identical to that given for NPY injections. The saline group than had unrestricted access to food and water during the 26 h of the experiment. 2) The NPY group received icv injections of NPY, 5 μg/injection, and also had unrestricted access to food and water throughout the experimental period. 3) The NPY yoked group received the same dose of NPY, 5 μg/injection, and also had unrestricted access to food and water during the 26 h of the experiment. 4) The saline without food group received icv saline icv injections and had no food available during the experiment but did have unrestricted access to water. The tissues were processed as in experiment 1, but a freezer accident destroyed the lipoprotein lipase studies. Data from the two halves of the experiment were combined for presentation.

GDP binding. The procedure, previously described (4), allows measurement of binding on a single rat. The mitochondria were obtained using differential centrifugation. Mitochondria (0.25 mg/ml) were incubated in a medium containing 100 mM sucrose, 20 mM K-NH₄Cl, and 1 mM K-EDTA, 10 mM choline chloride, 2 μM rotenone, (0.1 Ci/ml) [14C]sucrose (New England Nuclear), 0.1145 μM [3H]GDP (New England Nuclear), and 2 μM GDP (Sigma Chemical, St. Louis, MO). Total GDP binding was calculated from the known ratio of labeled to unlabeled compound. For modified Scatchard type analysis, the labeled GDP was combined with a range of unlabeled GDP concentrations (0–10 μM), and the ratios were used to estimate total GDP binding. The incubation was for 5 min with the reaction stopped by centrifugation and removal of supernatant. The mitochondrial pellet was then dissolved in NCS tissue solubilizer (Amer sham), and double-label scintillation counting was performed. GDP binding was assessed from 3H radioactivity with correction for trapped medium using [14C]sucrose. Total interscapular brown fat pad GDP binding was estimated by using the ratio of activity multiplied by volume of the marker enzyme cytochrome oxidase in the homogenate compared with the final mitochondrial preparation.

Cytochrome oxidase. An aliquot of homogenate and final mitochondrial preparation from each brown fat tissue sample was reserved for analysis. Cytochrome c oxidase activity was determined as described (4), utilizing the sample-induced oxidative change in optical density at 550 nM of a known amount of reduced cytochrome c.

Protein. An aliquot of brown adipose tissue homogenate was assayed for protein according to the method of Lowry (21).

Lipoprotein lipase. An aliquot of the homogenate of white adipose tissue was centrifuged at 20,400 g for 15 min, and the infranatant was removed and stored at −70°C until assay. Lipoprotein lipase was assayed using the method of Schotz et al. (30) as modified by Hietanen and Greenwood (14), with [14C]triolein as the substrate.

Serum metabolites. Glucose, lactate, pyruvate, β-hydroxybutyrate, acetoacetate, and free fatty acids were assayed by standard enzymatic techniques using an autoanalyzer (Technicon). This method permits assay of these metabolites on very small amounts of serum, thus improving serum availability for hormone assays.

Serum insulin and glucagon. Serum insulin and glu-
neuropeptide Y (NPY) and NPY with food intake yoked to control on brown fat thermogenic activity (GDP binding). A main effect of treatment was detected in analysis of variance (ANOVA) ($F = 6.522$, $P = 0.007$), and NPY groups were significantly different from control on post hoc testing with Scheffe’s test for the success of the yoking to the saline controls. These data provide no evidence for an effect on NPY on the

![Graph](image-url)

**TABLE 1. Body weight and tissue measures in experiment 1**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>NPY</th>
<th>NPY Yoked</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>10.2±1.4</td>
<td>36.5±3.0*</td>
<td>14.7±2.1</td>
</tr>
<tr>
<td>Body weight at cannulation, g</td>
<td>275.6±4.6</td>
<td>275.5±5.0</td>
<td>272.9±6.0</td>
</tr>
<tr>
<td>Body weight at death, g</td>
<td>274.5±8.8</td>
<td>301.3±10.8</td>
<td>274.9±7.9</td>
</tr>
<tr>
<td>White fat weight, mg</td>
<td>800±62</td>
<td>862±257</td>
<td>798±65</td>
</tr>
<tr>
<td>Brown fat weight, mg</td>
<td>197±94</td>
<td>297±30*</td>
<td>204±21</td>
</tr>
<tr>
<td>Brown fat protein content, mg/pad</td>
<td>28.6±4.5</td>
<td>22.8±2.4</td>
<td>20.5±4.7</td>
</tr>
<tr>
<td>Brown fat cytochrome oxidase, μmol/pad</td>
<td>23.2±3.5</td>
<td>13.9±1.7*</td>
<td>20.8±2.1</td>
</tr>
<tr>
<td>Brown fat cytochrome oxidase, μmol/mg protein</td>
<td>1.79±0.44</td>
<td>1.56±0.94</td>
<td>1.65±0.43</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of rats. * Significant difference from control ($P < 0.05$ on Scheffe’s post hoc test) after determination of significant effect of group in analysis of variance (ANOVA).
hormones and metabolites measured. There is no support from these data for the possibility that any serum metabolite or hormone measure, or the metabolic state that those measurements reflect, is responsible for the alterations in brown fat thermogenesis and white fat lipoprotein lipase activity that were observed.

The results of the second experiment (Figs. 4 and 5) show a clear effect of NPY in reducing GDP binding in both the fed and starved groups relative to both saline

![FIG. 2. GDP binding to brown fat mitochondria after NPY. Each point is mean of 3 measurements. Regression of bound-to-free (B/F) values on bound values is significantly (P < 0.0001) correlated for each plot. Plots are significantly different (P = 0.003).](http://ajpregu.physiology.org/)

![FIG. 3. Effect of NPY and NPY yoked to control on white fat lipoprotein lipase (LPL). Main effect of group on ANOVA (F = 16.516, P = 0.001);*NPY groups different from control but not from each other on Scheffe’s post hoc test.](http://ajpregu.physiology.org/)

![FIG. 4. Effect of NPY with and without food on GDP binding per mg brown fat mitochondrial protein. In a 2-factor ANOVA, there is a significant main effect of NPY (F = 7.512, P = 0.011), but no significant main effect of food status (F = 0.003, P = 0.958). Interaction term is not significant (F = 0.053, P = 0.82).](http://ajpregu.physiology.org/)

![FIG. 5. Effect of NPY with and without food on GDP binding per brown fat pad. In a 2-factor ANOVA, there is a significant main effect of NPY (F = 6.149, P = 0.02), but no significant main effect of food status (F = 2.602, P = 0.1). Interaction term is not significant (F = 0.027, P = 0.87).](http://ajpregu.physiology.org/)
term was not significant. In Fig. 5, GDP binding expressed as nanomoles per pad is shown. In this case, there does appear to be an effect of feeding compared with starvation on total GDP binding; however, the effect was not statistically significant by ANOVA. On the other hand, the effect of NPY relative to saline was significant. The interaction term was not significant. Overall, the binding data normalized to mitochondrial protein or estimated per pad indicates that NPY reduces brown fat thermogenesis as reflected by GDP binding. This reduction of thermogenesis by NPY does not depend on the fed status of the animal and can take place even when the animals have been starved for 26 h.

DISCUSSION

We had hypothesized that overfeeding induced by NPY, in analogy to overfeeding by cafeteria diet, would provoke brown fat thermogenesis in rats. We found instead that NPY treatment reduced brown fat thermogenesis. In addition, NPY treatment appeared to stimulate lipoprotein lipase activity in white adipose tissue. It appears that the NPY administration ivc can produce a coordinated effect leading to positive energy balance by inducing feeding, reducing thermogenesis in brown fat, and promoting storage of lipid fuels in white fat. Neither the NPY effect on brown fat nor the effect on white fat lipoprotein lipase has been previously reported. The observations are consistent with the finding of Egawa and colleagues (11) that NPY administration (250 and 500 pmol ivc) reduced brown fat sympathetic nerve firing rate. Further support comes from the report by Stanley and colleagues (34) that paraventricular nucleus administration of NPY increased food intake and increased food efficiency (weight gain to food intake ratio). The effects seen in brown fat and white fat do not require overfeeding. Furthermore, the effects on brown fat do not require any feeding at all. It appears, therefore, that the same stimulus, NPY given ivc, can produce coordinated effects on energy balance, all effects consistent with the production of positive energy balance.

While the coordinated effects produced by NPY were not consistent with results predicted from diet-induced thermogenesis, they were consistent with what Kray (5) has termed the "reciprocal" relationship between the food intake and thermogenesis found in a number of animal models of obesity and in some previous studies of central stimulation. Two animal models of genetic obesity demonstrate similar combined effects on food intake and peripheral energy metabolism. The genetically obese (ob/ob) mouse is hyperphagic but known to become obese when pair fed with lean litter mates (1). These mice have a thermogenic defect as manifest by failure to increase metabolic rate (38) or activate BAT (37, 15) in the cold like their lean mates. Current understanding of this animal points to a hypothalamic site of defect (16). The Zucker obese rat (fa/fa) is also hyperphagic but becomes obese when pair-fed with lean litter mates (6). They are cold intolerant and there is impaired whole body thermogenic response (23). An interpretation of these findings (22) has held that there is a central control abnormality expressed both neurobehaviorally and metabolically, and the hypothalamus is deficient in sympathetic nervous system activation, resulting in impairment of cold response and an inability to respond to stimuli such as overconsumption of nutrition.

Hypothalamic lesions can also produce a coordinated response involving reciprocal alterations in feeding and energy metabolism. The ventromedial hypothalamic (VMH)-lesioned rat has a prodigious appetite, but animals fed with controls become obese (7). The effect of VMH lesioning on energy expenditure in BAT is mildly controversial in its details but consistent with previous observations in its thrust. Hogan, Coscina, and Himms-Hagen (17) found that VMH lesioning increased food intake, produced obesity, and altered thermogenesis in BAT. Similar results were obtained when bilateral parasagittal hypothalamic knife cuts were made (9), disconnecting the VMH from the lateral hypothalamus. In addition, the lateral hypothalamus, the classical feeding center, also appears to contain controlling activities relevant to thermoregulation and thermogenesis. Lateral hypothalamic (LH) lesions result in decreased food intake (2) and increased whole body oxygen consumption (10). There is, therefore, evidence that lesions of the VMH increase food intake and decrease thermogenesis, whereas lesions of the complementary LH decrease food intake and increase thermogenesis.

Neuropharmacological stimuli can also effect energy intake as well as thermogenesis and metabolism. These studies have the virtue of involving a discrete stimulus applied to a discrete location but the disadvantage of pharmacological dosing. It is of particular interest that infusion of norepinephrine into the VMH causes obesity characterized by increased food intake, whereas brown adipose tissue shows increased triglyceride content while fatty acid synthesis is decreased, indicting that triglyceride turnover, and thus energy dissipation in brown adipose tissue, is decreased (33). Corticotropin-releasing factor (CRF) is another recent example of a neuropeptide with complementary effects on energy metabolism. Reduction of food intake appears to be a potent effect of CRF (24, 3), and increases in brown fat GDP binding in response to CRF have recently been reported for normal rats (18, 3). In each of these cases, one type of stimulus produces complementary alterations in feeding and energy expenditure through thermogenesis.

The physiological mechanisms by which central stimulation by NPY produced the observed effects are not yet fully identified. The most likely means by which central NPY would exert an effect on brown fat thermogenesis is by suppression of the sympathetic nervous system. The work of Egawa and colleagues provides evidence for this mechanism by showing a NPY-induced reduction in sympathetic nerve firing rate (11). The possibility of other mechanisms cannot be ruled out, but another explanation is not needed to explain the brown fat result. The effect of central NPY on white fat lipoprotein lipase activity is not as easily explained. Direct neural control of white fat activity is not widely accepted, although it remains a possibility. Induction of white fat lipoprotein lipase activity is normally associated with the action of insulin, and there is a report of increases in insulin after central injection of NPY in the rat (20), so...
it is tempting to find a role for insulin in these studies. There is little support in the data for such a temptation, however. In experiment 1, insulin rose in the NPY unrestricted group but not in the NPY yoked group, whereas lipoprotein lipase activity rose in both of these groups. It is possible that insulin was elevated transiently in the NPY yoked group and thereby produced an effect, but we have no evidence for that. One other mechanism that might contribute to the observed effects of NPY on both brown and white fat is stimulation of corticosterone. Central NPY has been reported to increase serum corticosterone levels (19), and corticosteroids have been reported to decrease brown fat thermogenesis (12) and increase white fat lipoprotein lipase activity (13). We did not measure corticosterone in this study. There is insufficient data to consider whether the time course and potency of corticosterone is consistent with the current data.

The current study of NPY effects does not utilize a specific site injection as yet for the production of the observed effects. Given the literature just cited, we believe, however, that it is likely that closely related sites do produce the spectrum of effects that we have observed. The previously cited observations of Stanley and colleagues (34) also favors the possibility of NPY producing all the currently reported effects at one site, because paraventricular nucleus administration of NPY increased food intake and also increased food efficiency. Furthermore, having made the observations detailed in this report, we believe that it is important to follow up on these observations by assessing the effect of NPY at specific brain sites on both feeding and peripheral energy metabolism. Furthermore, if these effects are seen after stimulation at a single site, it is important to determine whether these apparently connected effects can be separated by manipulations of diet, body energy stores, or metabolism or by brain manipulations. If the effects of NPY on feeding and peripheral energy metabolism are produced in different brain sites in response to the same neurotransmitter stimulation, it is important to understand the relationship of the different brain sites and what possibility there is for coordinate regulation.

In summary, NPY significantly increases feeding, decreases brown fat thermogenic activity, and increases white fat lipoprotein lipase activity. The NPY reduction of brown fat thermogenesis occurs when animals eat ad libitum and therefore overeat in response to NPY stimulation, when animals eat at a normal rate while being yoked to control group, and when animals eat nothing. The effects on white fat lipoprotein lipase activity occur at least when animals overeat in response to NPY or when the NPY stimulation occurs and food intake is yoked to control. The metabolic changes produced by NPY and brown fat and white fat are unrelated to changes in serum glucose, insulin, glucagon, lactate, pyruvate, β-hydroxybutyrate, acetoacetate, triglyceride, glycerol, or free fatty acids. We conclude that central stimulation by NPY provokes a coordinated effect on energy balance, simultaneously increasing food intake, decreasing facultative thermogenesis in brown fat, and increasing fat storage in white fat.

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