Activation of the NPY Y5 receptor regulates both feeding and energy expenditure

Hwa, Joyce J., Melanie B. Witten, Patricia Williams, Lorraine Ghiaudi, J un Gao, Brian G. Salisbury, Deborah Mullins, Fozia Hamud, Catherine D. Strader, and Eric M. Parker. Activation of the NPY Y5 receptor regulates both feeding and energy expenditure. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1428–R1434, 1999.—Intracerebroventricular (ICV) administration of neuropeptide Y (NPY) has been shown to decrease energy expenditure, induce hypothermia, and stimulate food intake. Recent evidence has suggested that the Y5 receptor may be a significant mediator of NPY-stimulated feeding. The present study attempts to further characterize the role of NPY Y5-receptor subtypes in feeding and energy expenditure regulation. Satiated Long-Evans rats with temperature transponders implanted in the interscapular brown adipose tissue (BAT) displayed a dose-dependent decrease in BAT temperature and an increase in food intake after ICV infusion of NPY. Similar effects were induced by ICV administration of peptide analogs of NPY that activate the Y5 receptor, but not by analogs that activate Y1, Y2, or Y4 receptors. Furthermore, ICV infusion of the Y5 selective agonist D-[Trp32]-NPY significantly reduced oxygen consumption and energy expenditure of rats as measured by indirect calorimetry. These data suggest that the NPY Y5-receptor subtype not only mediates the feeding response of NPY but also contributes to brown fat temperature and energy expenditure regulation.

brown fat; hypothermia; intracerebroventricle; indirect calorimetry; obesity

HYPOTHALAMIC NEUROPEPTIDE Y (NPY) plays a critical role in the regulation of both energy intake and energy expenditure. Numerous studies have demonstrated the potency of this 36-amino acid peptide as an orexigenic agent when administered centrally (1, 25). Central administration of NPY has also been shown to decrease sympathetic nervous system activity (9) and to suppress thermogenesis in brown adipose tissue (BAT), a primary area involved in the regulation of rodent energy expenditure (3). These coordinated effects of NPY on energy homeostasis lead to a state of positive energy balance and weight gain in the form of fat (24).

By measuring rectal body temperature, a physiological response that may reflect whole body energy expenditure, several studies reported that NPY can cause whole body hypothermia (6, 7, 20). NPY can elicit feeding responses after administration into many hypothalamic nuclei such as perifornical, ventromedial, paraventricular, arcuate, and preoptic nuclei, with the strongest effect found in the perifornical hypothalamus. In comparison, NPY-induced hypothermia can be seen after administration of the peptide into preoptic, paraventricular, and arcuate nuclei of the hypothalamus (7, 15). The fact that NPY infusion elicited only feeding but not hypothermia in the perifornical hypothalamus suggests that the feeding and hypothermic responses can be mediated by different hypothalamic nuclei (7). Bouali et al. (5) further demonstrated that the NPY-induced hypothermia was mediated by pertussis toxin-sensitive G protein-linked NPY receptor(s). However, the identity of the NPY-receptor subtypes that mediate the effects of NPY on energy homeostasis remains elusive. To date, at least six distinct G protein-linked NPY receptors have been cloned and characterized (4). With the use of in situ hybridization techniques, Y1-, Y2-, and Y5-receptor mRNA have been detected in numerous hypothalamic nuclei, including paraventricular, arcuate, and perifornical nuclei (12, 18). The pharmacological properties of the receptor subtype(s) mediating NPY-induced feeding are most similar to the pharmacological properties of the Y5 receptor (11), although the Y1 receptor also is involved in the control of feeding behavior (16, 26). The pharmacological properties of the NPY-receptor subtype(s) mediating energy expenditure regulation have not been rigorously studied. The main focus of this study is to administer NPY and its analogs by intracerebroventricular (ICV) infusion to evaluate the NPY receptor subtype(s) involved in the regulation of energy expenditure.

The discovery that BAT is an important site of thermogenesis in the rat indicates that BAT plays a significant role in modulating energy expenditure (8). Since BAT thermogenesis induces energy dissipation as heat, the change in BAT temperature may indicate relative thermogenic activities in BAT. The present study uses temperature transponders implanted in the interscapular BAT to measure the change of temperature in the BAT after ICV administration of NPY analogs. We characterized the receptor(s) mediating NPY-induced suppression of BAT temperature while concurrently measuring NPY-induced feeding behavior in free-roaming rats. To identify the receptor subtype(s) involved in NPY-mediated energy regulation, we correlated the in vitro binding and functional activities of several NPY analogs at the cloned rat Y1, Y2, Y4, and Y5 receptors with their in vivo effects on feeding and BAT temperature in ICV-cannulated rats. In addition,
Y5 receptor-mediated energy expenditure regulation was studied by indirect calorimetry. Our data indicate that activation of central Y5 receptors not only increases feeding but also decreases brown fat temperature and energy expenditure in rats.

MATERIALS AND METHODS

Animals. Adult male Long-Evan rats (250–300 g; Charles River, MA) were maintained in individual cages at 22°C on a 12:12-h light-dark cycle with lights on at 0400. Rats had free access to food (Teklad Lab Rodent Chow #8604, Bartonville, IL) and water. All studies were conducted in an American Association for Accreditation of Laboratory Animal Care accredited facility following protocols approved by the Animal Care and Use Committee of Schering-Plough Research Institute. The procedures were performed in accordance with the principles and guidelines established by the NIH for the care and use of laboratory animals.

Surgery. Rats were anesthetized by intramuscular injection of a mixture of ketamine and xylazine (100:10 mg/kg). A 22-gauge stainless steel cannula was stereotaxically implanted. The stereotaxic coordinates for the right lateral ventricle were obtained from the atlas of Paxinos and Watson (19). Briefly, the incisor bar was adjusted until the height of lambda and bregma skull points were equal. This flat-skull position was achieved when the incisor bar was lowered ~3.9 mm below the interaural line. The stereotaxic coordinates for ICV cannulation were 1.0 mm posterior to bregma, 1.5 mm lateral to midline, and 2.6 mm ventral to dura, with the ICV infusion needle extended to 3.6 mm ventral to dura. Animals were also inserted with temperature transponder probes (Implantable Programmable Temperature Transponder, Bio-Medic Data System, Seaford, DE) in the interscapular BAT area. In some of the rats intraperitoneal telemetry temperature implants were placed in the abdominal cavity to monitor core body temperature changes. After a 3-wk recovery period, all animals were tested for cannula placement by ICV infusion of NPY (0.3 nmol). Animals demonstrating a profound feeding effect (~2.0 g) within 60 min of infusion were retained for the study.

Peptides and ICV infusion protocols. Human (h) or rat (r) NPY, h NPY free acid, hr NPY-(2–36), hr NPY-(13–36), h pancreatic polypeptide (PP), rPP, h peptide YY (PYY)-(3–36), C2-NPY, and h-[

RESULTS

Effects on binding and cAMP assays. NPY potently binds and activates the cloned rat Y1, Y2, and Y5 receptors but is a much less effective activator of the Y4 receptor (Table 1). Both hPP and rPP potently activate the rat Y4 receptor in cAMP assays (Table 1), whereas hPP is 87-fold more potent than rPP in activating the rat Y5 receptors in cAMP assays. Hence, hPP is a Y4- and Y5-receptor agonist, whereas rPP only activates the Y4 receptors. NPY-(2–36) and PYY-(3–36) are Y2- and Y5-receptor agonists, whereas C2-NPY and NPY-(13–36) selectively activate the Y2 receptor (Table 1). Replacing the Thr32 of NPY with D-Trp completely abolishes the Y1 and Y2 activities of NPY, while retaining some of the Y5 activity. d-[Trp32]NPY is a very selective Y5 agonist, with Kd and EC50 values of 34 and 25 nM, respectively, at the cloned rat Y5 receptor (Table 1).

Effects on feeding. ICV administration of NPY elicits robust feeding behavior in satiated cannulated rats in a dose-dependent manner [half-maximal dose (ED50) = 0.32 nmol; Fig. 1A]. In contrast, ICV administration of the inactive COOH-terminal free acid form of NPY does not induce food intake in the rats (Fig. 1A) (25). The Y4- and Y5-receptor agonist hPP also induces a dose-dependent increase of feeding behavior (ED50 = 0.5
Table 1. Summary of agonist potencies for selected peptides at cloned rat NPY receptors

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Rat Y1* EC50</th>
<th>pEC50</th>
<th>Rat Y2* EC50</th>
<th>pEC50</th>
<th>Rat Y4† EC50</th>
<th>pEC50</th>
<th>Rat Y5† EC50</th>
<th>pEC50</th>
<th>Temp.2 Food2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY</td>
<td>0.359±0.08</td>
<td>9.540±0.10</td>
<td>2.13±0.51</td>
<td>8.707±0.10</td>
<td>6.562±0.03</td>
<td>8.837±0.03</td>
<td>3.63±0.2</td>
<td>8.453±0.03</td>
<td></td>
</tr>
<tr>
<td>NPY-(2—36)</td>
<td>5.73±1.4</td>
<td>8.283±0.12</td>
<td>4.73±2.5</td>
<td>8.453±0.22</td>
<td>1011±128</td>
<td>6.003±0.06</td>
<td>8.43±0.9</td>
<td>8.083±0.05</td>
<td></td>
</tr>
<tr>
<td>NPY-(3—36)</td>
<td>66.2±7.8</td>
<td>7.194±0.05</td>
<td>6.63±2.7</td>
<td>8.243±0.18</td>
<td>418±57</td>
<td>6.393±0.05</td>
<td>23.7±3.0</td>
<td>7.633±0.06</td>
<td></td>
</tr>
<tr>
<td>NPY-(13—36)</td>
<td>141±45</td>
<td>6.907±0.14</td>
<td>5.9±1.2</td>
<td>8.253±0.09</td>
<td>279±19</td>
<td>6.562±0.03</td>
<td>1.617±0.14</td>
<td>8.837±0.03</td>
<td></td>
</tr>
<tr>
<td>α-[Trp32]-NPY</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>25.3±1.9</td>
<td>7.593±0.10</td>
<td></td>
</tr>
<tr>
<td>PYY-(3—36)</td>
<td>193±65</td>
<td>6.777±0.15</td>
<td>1.4±0.3</td>
<td>8.873±0.08</td>
<td>518±101</td>
<td>6.352±0.09</td>
<td>8.83±1.4</td>
<td>8.063±0.05</td>
<td></td>
</tr>
<tr>
<td>C2-NPY</td>
<td>811±172</td>
<td>6.111±0.11</td>
<td>16.0±6.2</td>
<td>7.933±0.22</td>
<td>682±93</td>
<td>6.173±0.05</td>
<td>739±7.4</td>
<td>6.144±0.04</td>
<td></td>
</tr>
<tr>
<td>hPP</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>0.086±0.03</td>
<td>10.103±0.12</td>
<td>1.13±0.14</td>
<td>8.96±0.06</td>
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<tr>
<td>rPP</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>&gt;1.00</td>
<td>&lt;6.00</td>
<td>0.051±0.01</td>
<td>10.323±0.10</td>
<td>98.0±10.8</td>
<td>7.023±0.05</td>
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</tr>
</tbody>
</table>

Values are means ± SE (superscripts denote sample size). NPY, neuropeptide Y; hPP, human pancreatic polypeptide; rPP, rat polypeptide.

*CHO-K1 cells; †HEK-293 cells; <, decreased brown fat temperature; ↑, increased food intake; →, no effect.

Fig. 1. Differential effects of neuropeptide Y (NPY) vs. NPY-free acid (A), human (h) pancreatic polypeptide (PP) vs. rat (r) PP (B), NPY-(2—36), peptide YY (PYY)-(3—36), NPY-(13—36), and C2-NPY (C), and α-[Trp32]-NPY (0.1–3 nmol; D) on 2 h food intake after intracerebroventricular (ICV) infusion in cannulated Long-Evans rats. Values are means ± SE (n = 6–10). *Values significantly different from saline control group (P < 0.05).
nmol), whereas the Y4-selective agonist rPP did not cause any significant changes in food intake (Fig. 1B). NPY analogs such as NPY-(2–36) and PYY-(3–36) both stimulate food intake (ED50 = 0.34 and 0.29 nmol, respectively; Fig. 1C). However, the feeding responses induced by either NPY-(13–36) or the Y2-selective agonist C2-NPY are not statistically different from that of the saline control group (Fig. 1C). High doses of D-[Trp32]NPY elicit a modest but significant increase in food intake 2 h after the ICV infusion (ED50 = 1.45 nmol; Fig. 1D).

Effects on brown fat temperature. With the use of the BAT temperature transponders, we have detected a significant increase of BAT temperature after bolus subcutaneous injection of the b3 agonist ICI198157, which selectively increases brown fat thermogenesis measured by GDP binding to BAT mitochondria (13). In cold-adapted rats, Howe et al. (13) reported that subcutaneous injection of 15 mg/kg of ICI198157 significantly increased core body temperature. In this study, we used a 100-fold lower dose of ICI198157, which selectively increases BAT temperature versus core body temperature (Fig. 2), suggesting that the BAT temperature transponder can sensitively detect BAT thermogenesis in vivo. ICV administration of NPY significantly decreases BAT temperature beginning at 30 min postinfusion and reaching maximum at about 60 min postinfusion. Both brown fat and core body temperature decline in a dose-dependent manner after ICV infusion of NPY (Figs. 3 and 4A). The selective Y4 agonist rPP did not elicit any significant changes in brown fat temperature. In contrast, the Y4- and Y5-receptor agonist hPP significantly reduces BAT temperature at 60 min postinfusion (Fig. 4B). NPY analogs such as NPY-(2–36) and PYY-(3–36) both significantly lower BAT temperature 1 h after the ICV infusion (Fig. 4C). However, the Y2-selective agonists (C2-NPY) and NPY-(13–36) did not have any effect on BAT temperature (Fig. 4C). Furthermore, administration D-[Trp32]-NPY into the lateral ventricle of rats leads to significant reduction of BAT temperature at all doses tested (Fig. 4D).

Effects on energy expenditure. ICV administration of the Y5-selective agonist D-[Trp32]-NPY (3 nmol) causes a significant 30% decrease in V˙O2 and energy expenditure compared with saline control rats during the first hour postinfusion period (Fig. 5, A and B). However, the respiratory quotient of D-[Trp32]-NPY-treated rats is not significantly different from that of the saline controls (Fig. 5C).

Fig. 2. Comparison of changes of BAT vs. core body temperature 1 h after subcutaneous injection of a b3 agonist (ICI198157; 0, 0.1, and 0.3 mg/kg). Values are means ± SE (n = 8). dt, temperature difference between treated and previous saline control data for each animal. *Values significantly different from saline control group (P < 0.05).

Fig. 3. Correlation of changes of BAT vs. core body temperature 1 h after ICV infusion of NPY (0, 0.3, 1 nmol). Values are means ± SE (n = 8). *Values significantly different from saline control group (P < 0.05).

DISCUSSION

NPY modulates whole body energy balance by regulating food consumption, energy expenditure, and the metabolic processes that determine the balance between fat mobilization and storage. These physiological effects of NPY are mediated by interaction with at least six distinct G protein-coupled receptors (designated Y1-Y6) (4). The present study demonstrates that both the feeding and BAT hypothermic effects induced by central administration of NPY analogs are most consistent with activation of Y5 receptors.

Several lines of evidence from our data support the notion that central activation of Y5 receptors not only stimulates food intake but also decreases BAT temperature. First, ICV infusion of the Y4/Y5 agonist hPP, but not the selective Y4 agonist rPP, stimulates feeding and BAT hypothermia. This suggests that the Y5-derived activity of hPP is responsible for such effects. Second, the Y2/Y5 agonists NPY-(2–36) and PYY-(3–36) increase food intake and decrease BAT temperature. Because the Y2 selective agonist C2-NPY has no effect on food intake or BAT temperature, the activity of Y2 and Y5 agonists must be due to Y5-receptor activation.
Third, the Y5 selective agonist d-[Trp32]-NPY induces feeding and significantly reduces BAT temperature in satiated rats. With the use of an indirect calorimetric method, we demonstrated that immediately after central administration of a selective Y5 agonist d-[Trp32]-NPY (3 nmol) rats experienced a significant reduction of $\dot{V}O_2$ and energy expenditure without affecting respiratory quotient. The transient 30% reduction of energy expenditure correlates well with the decrease of BAT temperature after central administration of d-[Trp32]-NPY and suggests that the decrease in BAT temperature partly reflects the reduction of energy expenditure. Collectively, our data support the hypothesis that central activation of Y5 receptor leads to increases in food intake and decreases of BAT temperature and energy expenditure.

The lack of selective Y1 agonists precludes a fair evaluation of the role of the Y1 receptor in feeding or BAT temperature regulation. The fact that PYY-(3—36), hPP, and d-[Trp32]-NPY all induce feeding and BAT hypothermic effects, despite being inactive at the cloned Y1 receptor (Table 1), suggests that Y1 receptors are not essential for eliciting feeding and BAT hypothermic effects. However, recent reports of Y1 and Y5 receptor knockout mice indicate that both Y1 and Y5 receptors are involved in feeding behavior. Y5-deficient mice had significantly lower NPY, hPP, or PYY-(3—36)—induced food intake (16), whereas Y1-deficient mice showed reduced fasting-induced feeding but not NPY-induced feeding (21). Further studies with selective Y1-receptor agonists and antagonists will be required to determine...
if activation of Y1 and Y5 receptors are both involved in NPY-dependent energy expenditure regulation.

BAT not only plays an important role in nonshivering-induced thermogenesis, but it also plays a significant role in diet-induced thermogenesis (22). Therefore, increasing food intake should cause BAT hyperthermia and elevated energy expenditure. However, our study has shown that NPY and Y5-selective peptides elicit feeding as well as BAT hypothermia, suggesting that the BAT hypothermic effect is not secondary to the feeding effect. The factors contributing to the BAT hypothermia observed after central administration of NPY analogs in the present study remain to be determined. Central administration of NPY has been shown to decrease sympathetic nervous system activity (9). The sympathetic nervous system modulates energy expenditure by increasing thermogenesis through activation of b3 receptors and elevation of uncoupling protein activity in BAT. BAT thermogenic activity is reduced after central NPY administration (3). However, not only BAT but also core body temperatures are both substantially reduced after central administration of NPY in our study. Therefore, some other mechanism such as changing regional blood flow in BAT or other subcutaneous area (14, 28) may also contribute to the simultaneous decrease in BAT and core body temperature.

Hypothalamic NPY plays a fundamental role in the regulation of energy balance through its orexigenic activity and profound metabolic effects. Central administration of NPY can promote a state of positive energy balance by coordinating energy intake and expenditure through the regulation of feeding and thermogenesis. Comparing the in vitro activities of selective NPY analogs with their in vivo functions, we conclude that central activation of Y5 receptors can stimulate BAT hypothermia as well as food intake. The significant 30% decrease of energy expenditure elicited by central administration of Y5-selective agonist d- [Trp32]NPY
further supports the role of NPY and the Y5 receptor in the regulation of energy balance.

Perspectives

NPY plays a critical role in the successful energy regulation of normal individuals as well as the dysregulation of energy balance that characterizes obesity. For example, hypothalamic NPY peptide and mRNA levels are increased after fasting in normal rats; however, NPY mRNA levels are higher in genetically obese mice and rats without fasting (2, 23, 27). Central administration of NPY increases food intake and decreases energy expenditure in satiated rats (3, 25), suggesting that NPY can promote positive energy balance. Both Y5 and Y1 receptors seem to play an important role in the feeding behavior. The pharmacological properties of the Y5 receptor most closely correspond to the pharmacological properties of the receptor that mediates feeding induced by exogenous NPY and NPY analog (11), whereas the Y1 receptor may play a substantial role in fasting-induced feeding (21). However, the NPY-receptor subtype, which mediates the reduction of energy expenditure induced by NPY, has not been characterized. Our data indicate that central Y5-receptor activation is responsible for the observed feeding as well as the energy expenditure effects of NPY.

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