Peptide YY (3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys

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Abstract

Peptide YY$_{3-36}$ [PYY(3-36)], a gastrointestinal peptide that is released into the circulation in response to ingesting a meal, has recently been suggested to play a role in controlling food intake. PYY(3-36) has been reported to inhibit food intake following peripheral administration in rodents and in human subjects. To more fully characterize the potential feeding actions of PYY(3-36), we examined the ability of a dose range of PYY(3-36) (0.3-3.0 nmol/kg) to affect liquid gastric emptying and daily 6 hr food intake in male rhesus monkeys. Intramuscular (IM) PYY(3-36) produced a dose related inhibition of saline gastric emptying that was maximal at a dose of 3 nmol/kg. IM PYY(3-36) administered prior to daily 6 hr food access produced significant feeding reductions at doses of 1 and 3 nmol/kg. Analyses of the patterns of food intake across the 6 hr period of food access revealed that PYY(3-36) increased the latency to the first meal and reduced average meal size without altering meal number. Although single doses of PYY(3-36) reduced intake, a suppressive effect on food intake was not sustained over multiple administrations across successive days. Together, these data suggest that PYY(3-36) has the ability to reduce food intake in acute test situations in non-human primates. Whether this is a physiological action of the endogenous peptide remains to be determined.

Key words: satiety, meal patterns, gut peptides
Consumed nutrients excite the release of a variety of gastrointestinal peptides that serve as feedback signals for coordinating nutrient movement through the gastrointestinal tract, absorption and metabolism. Different peptides have different sites and patterns of release. Roles for some of those peptides in the controls of food intake have been proposed and/or documented.

Recently, PYY(3-36), a peptide has been demonstrated to reduce food intake in rodents and man. Batterham and colleagues (4) reported significant suppression of food intake in mice and rats in response to peripheral bolus PYY(3-36) administration and significant decreases in food intake in human subjects in response to PYY(3-36) infusions. An action of peripheral PYY(3-36) through arcuate nucleus Y2 receptors was proposed. The Y2 receptor is a presynaptic inhibitory receptor and PYY(3-36) has been demonstrated to have relative specificity for this NPY receptor subtype (13). Consistent with this proposal, PYY(3-36) was shown to inhibit food intake when injected into the arcuate nucleus in intact but not in Y2−/− mice and PYY(3-36) increased activity in POMC neurons through decreasing the frequency of inhibitory post-synaptic currents, indicative of a decrease in GABA release. Since arcuate NPY neurons exert a tonic GABAergic inhibitory influence on arcuate POMC neurons, this action of PYY(3-36) was interpreted to support a feeding inhibitory action of PYY(3-36) through inhibition of arcuate NPY neurons resulting in increased activity in arcuate POMC neurons. Recent work has demonstrated feeding inhibitory actions of PYY(3-36) on mice lacking either the melanocortin 4 receptor (11) or POMC (5), demonstrating that increased melanocortin activity is not necessary for the feeding inhibitory action of PYY(3-36).
Other investigators have not been able to replicate the feeding inhibitory actions of PYY(3-36). A recent report combining results from multiple laboratories (including ours), presented negative data on the ability of PYY(3-36) to inhibit food intake and reduce body weight in rodents across a range of doses and testing paradigms (24). The reasons for the differences in results are not clear.

In an effort to further characterize the potential role of PYY(3-36) in the controls of food intake and gastrointestinal function, we evaluated the ability of PYY(3-36) to inhibit food intake in nonhuman primates following intramuscular bolus administration. We chose our dose range based on preliminary studies evaluating the ability of PYY(3-36) to inhibit the gastric emptying of a non-nutrient gastric load.

Methods

All procedures were approved by the Animal Care and Use Committee of Johns Hopkins University. Subjects were 4 male rhesus monkeys weighing between 10 and 14 kg. Monkeys were individually housed and maintained on a 12/12h light-dark cycle (lights on at 07:00). Water was available ad libitum except during gastric emptying experiments. Food in the form of 1 gm food pellets (Bio-Serv) was available in response to lever presses on a fixed ratio (FR) reinforcement schedule beginning at 12:00 PM for 6 hours per day. Monkeys quickly adapt to this feeding schedule and maintain normal rates of weight gain. Chronic indwelling gastric cannulas were surgically implanted into the most dependent portion of the stomach along the greater curvature as previously described (16, 17). The cannulas permitted the infusion into and withdrawal of liquids from the stomach. Monkeys wore a vest that had a
multiflexible stainless steel cable attached at the back. The gastric cannulas ran through the cable and out the back of the cage allowing remote access to the gastric cannula for gastric infusions and withdrawal of gastric contents.

Gastric emptying experiments were carried out between 9:00 and 11:00 AM after an overnight fast. We examined the effect of a dose range (0, 0.3, 0.6, 1 and 3 nmol/kg) of PYY(3-36) on the gastric emptying of a saline (0.9% NaCl) test meal. 150 ml of saline containing phenol red were infused into the stomach at a rate of 25 ml/min over a 6 min period. Ten minutes following the termination of the infusion, the stomach was emptied and rinsed with distilled water. PYY(3-36) doses or saline vehicle were administered intramuscularly (IM) 15 min prior to the infusion of the saline test meals. There were at least 2 days without an injection of PYY(3-36) between each gastric emptying test. Volume of the original test meal remaining was ascertained using the dye dilution technique of Hunt and Spurrel (12). Gastric volumes remaining following the various doses of PYY(3-36) were compared using repeated measures ANOVA and planned t comparisons.

The effects of PYY(3-36) on food intake were examined in the same 4 rhesus monkeys. Fifteen minutes prior to the normal 6 hr period of food access (12:00 – 18:00), monkeys received an IM injection of PYY(3-36) (0.3, 1 and 3 nmol/kg) or saline vehicle. One gram food pellets were available in response to lever presses on an FR schedule ranging from an FR3 to an FR5. The particular reinforcement schedule was chosen for each individual monkey to prevent them from spilling or pouching the pellets without altering their feeding pattern. The timing of each pellet delivery was recorded by computer using Med Associate software and intake data were subsequently analyzed.
for total intake at hourly intervals, meal patterns and latency to the first meal. Meals were defined as the acquisition of at least 5 pellets preceded and followed by a period of at least 10 min without feeding. These meal parameters accounted for greater than 95% of the pellets. A saline baseline day preceded each dose of PYY(3-36). For analyses, the mean of the three baseline levels for each monkey served as the saline control values. Data for hourly intakes and for each meal pattern variable were analyzed by repeated measures ANOVA, analyses of simple effects and planned t comparisons.

The effect of repeated administration of the largest PYY(3-36) dose (1 nmol/kg) over 4 days on daily food intake was also examined. For these experiments, vehicle was administered on day 0 and 1 nmol/kg PYY(3-36) was administered for 4 consecutive days. Intakes on each pf the PYY(3-36) days was compared with intake on the vehicle day. We have employed this paradigm previously to examine the feeding effects of repeated administration of a CCK analog (19). Data were analyzed for hourly intakes and meal pattern as described above.

Results

As demonstrated in Figure 1, PYY(3-36) resulted in a dose related suppression of saline gastric emptying, F(4,12) = 36.811, p < .0001. All doses resulted in significant increases in the volume of the original saline test meal remaining at the 10 min post fill time point (p < .01) and gastric emptying was essentially completely prevented at the 3 nmol/kg dose.
**Food Intake**

PYY(3-36) also inhibited 6 hour daily food intake (Figure 2). There was an overall effect of PYY(3-36) dose, $F(3,9) = 6.355$, $p < .02$. The inhibition of overall intake was significant at both the 1 and 3 nmol/kg doses ($p < .01$) and the magnitude of inhibition was not different at these doses. Figure 3 shows the hourly pattern of intake at the vehicle, 1 nmol/kg and 3 nmol/kg doses. Analyses of simple effects indicated that intake was significantly inhibited by both doses of PYY(3-36) at the 1, 2, 4, 5 and 6 hr time points. Meal pattern analysis (Table 1) revealed a number of effects of PYY(3-36). Although there was significant variability in the effect of PYY(3-36) on the latency to the first meal, latency increased with increasing dose and this effect was significant at the 3 nmol/kg dose (paired t comparison $p < .05$). Average meal size was also decreased in response to PYY(3-36), $F(3,9) = 3.934$, $p < .05$ with significant reductions at both the 1 and 3 nmol/kg doses ($p < .05$). Meal frequency was not affected by PYY(3-36) nor was the size of the first meal of the feeding period.

Repeated daily administration of 1 nmol/kg PYY(3-36) did not result in sustained effects on daily 6 hour food intake. There were no significant suppressions of total food intake on days 2 through 4. Figure 4 shows the comparison of vehicle intake with the mean hourly intakes combined over days 2-4 of PYY(3-36) administration. Intake was only reduced at the 1 hr time point but this reduction was not significant. This decrease was made up in the subsequent 5 hour of the feeding period. There were no significant effects on meal parameters through this period.

**Discussion**
Although there is some controversy over the findings, PYY(3-36) has been previously demonstrated to induce an acute decrease in food intake in rodents and human subjects (2-4, 6). The data presented here extend these findings to non human primates and demonstrate that acute PYY(3-36) administration reduces daily food intake in rhesus monkeys at doses that suppress gastric emptying. The feeding suppressive effects of PYY(3-36) were manifested as increased latencies to initiate daily feeding and as consistent overall decreases in meal size. Repeated PYY(3-36) administration across days did not result in maintained decreases in food intake.

PYY and PYY(3-36) are found throughout the gastrointestinal tract with highest concentrations found at distal small and large intestinal sites (20). PYY(3-36) is released from L cells in response to food ingestion (20). Studies in dogs have demonstrated that plasma levels of PYY gradually rise following meal initiation, reaching a peak at about 60 min and high levels are maintained for a number of hours following a meal (20). Such data are not yet available on the patterns of PYY(3-36) secretion in response to a meal but PYY and PYY(3-36) do seem to be coreleased suggesting that the pattern of PYY(3-36) release is similar to that of PYY (10). A gradual increase and sustained levels of post meal PYY(3-36) would suggest that it may be involved in delaying the time to a subsequent meal and affecting feeding over a longer time period. The data from the current study are consistent with the potential role for PYY(3-36) suggested from the normal dynamics of PYY(3-36) release. Exogenous PYY(3-36) delayed feeding onset and had lasting effects on meal sizes.

The ability of PYY(3-36) to inhibit gastric emptying is consistent with the suggested role of PYY and its derivatives in the ileal brake (14, 22, 25). In the present
study, PYY(3-36) induced a dose related suppression of liquid gastric emptying that was maximal at a dose of 3 nmol/kg. Actions of peripherally administered PYY on gastric emptying have long been noted (21) and this effect was unlikely due to interactions with Y1 or Y5 sites since peripheral NPY did not result in similar inhibitions of gastric emptying (1). Further supporting this interpretation, recent work comparing the gastric inhibitory potency of full length PYY with that of PYY(3-36), demonstrated that PYY(3-36) was an order of magnitude more potent than PYY(1-36) (7). Such a pharmacological profile is consistent with action at the Y2 site, the NPY receptor subtype for which PYY(3-36) has the greatest affinity (13).

The relationship between the gastric inhibitory and feeding inhibitory actions of PYY(3-36) is not clear. Delayed gastric emptying itself can reduce food intake and multiple treatments that reduce food intake also reduce gastric emptying. However, the contribution of the gastric inhibitory effects of such treatment to the feeding inhibition is not always straightforward. For example, CCK inhibits gastric emptying and the magnitude of a CCK induced inhibition of food intake can be demonstrated to be significantly increased under conditions in which the emptying of a gastric load is inhibited by CCK(18). Although such a contribution can be demonstrated, CCK induced feeding inhibition can occur in sham feeding tests, a condition in which gastric feedback is essentially eliminated (9). Batterham et al had demonstrated inhibition of food intake in rodents at doses that did not alter the percent of solid food remaining in the stomach at a 3 hr time point, suggesting that inhibition of gastric emptying was not a mechanism underlying the PYY(3-36) induced inhibition of food intake (4).
The mediation of the feeding inhibitory actions of PYY(3-36) in the primate are not clear. Batterham and colleagues have suggested a hypothalamic site of action for PYY(3-36) induced feeding inhibition in the rodent (4). Electrophysiological data support an inhibitory action of PYY(3-36) on NPY neurons resulting in an overall reduction in NPY activity and the release of a GABAergic input on POMC neurons. Recent data has suggested that increased activity in POMC neurons may not be necessary for the feeding inhibitory actions of PYY(3-36) as mice lacking POMC (5) or melanocortin receptors (11) have been reported to be responsive to the feeding inhibitory action of PYY(3-36). Other gut peptides can affect food intake through vagal or hindbrain actions (15, 23). Although vagal mediation of the feeding inhibitory actions of PYY(3-36) are yet to be assessed, recent data suggest that hindbrain sites may not be involved as lesions of the area postrema, rather than eliminating, seem to enhance the feeding suppressive actions of PYY(3-36) (8). No mechanistic studies have been done in primate species.

The feeding suppressive actions of PYY(3-36) in the primate appear to be short lived. Inhibition on food intake were obtained on the first day of a multiple day course of PYY(3-36), but intakes on subsequent days were not significantly reduced relative to intakes on the baseline day preceding the first PYY(3-36) injection. This finding is in contrast with the results of repeated administration of PYY(3-36) in rats as reported in the original Batterham et al study in which significant suppressions were maintained over a 7 day course of treatment (4). However, Challis et al have recently reported that although acute PYY(3-36) reduced food intake (5), there were no sustained effects through a 7 day treatment trial with cumulative food intake indistinguishable between
saline and PYY(3-36) treated mice. Although PYY(3-36) reduces food intake in both lean and obese human subjects in acute experiments (3, 4), its ability to reduce intake following repeated administration has yet to be assessed. The current data from nonhuman primates suggest that PYY(3-36) will not maintain the ability to reduce intake over multiple days in human subjects.

We employed an intramuscular route of administration of PYY(3-36) in these studies for a number of reasons. Prior use of this route of administration in studies examining the ability of a CCK analog to reduce food intake yielded positive and reliable results (19). Monkeys tolerate IM injections well and can be trained to come to the front of the cage for injections. Use of this route of administration also eliminates the need for additional surgeries that would be required for chronic cannulations. Rodent studies on the feeding inhibitory actions of PYY(3-36) with both positive and negative results have characterized the actions of multiple routes of administration and there does not appear to be any clear pattern. Positive data have been reported following intraperitoneal (IP) and intravenous (IV) administration (4, 6, 11). Negative findings have been obtained with IP, subcutaneous (SC) and IV routes of administration (24). Acute IP injections of a dose of 0.3 µg/kg have been reported to produce physiological postprandial plasma levels in rats (4). Plasma levels were not assessed in the present studies but our dose range of 0.3 to 3 nmol/kg would likely produce plasma levels that would attain and surpass normal postprandial levels of PYY(3-36).

In summary, the present data demonstrate that in rhesus monkeys PYY(3-36) exerts a dose related inhibition on liquid gastric emptying over a dose range of 0.3 to 3 µ/kg. PYY(3-36) also inhibits food intake at the upper end of this dose range. The
effect on food intake does not appear to be dose related; the magnitude and pattern of suppression at the 1 and 3 µ/kg doses were similar. The PYY(3-36) feeding inhibition is expressed as an increased latency to the first meal and an overall decrease in meal size. Effects on food intake were not sustained over multiple days of PYY(3-36) administration. Further work is needed to determine if the inhibition of food intake is a physiological function of endogenous PYY(3-36) in nonhuman primates.
Acknowledgement:

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### Table 1

**PYY(3-36) - Induced Alterations in Meal Patterns**

<table>
<thead>
<tr>
<th>PYY(3-36) Dose</th>
<th>Meal Size (# pellets)</th>
<th>Meal Number</th>
<th>1st Meal Size (# pellets)</th>
<th>Latency to 1st Meal (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>24.3 ± 3.6</td>
<td>7.3 ± 0.9</td>
<td>31.4 ± 8</td>
<td>0.23 ± 0.10</td>
</tr>
<tr>
<td>0.3 nmol/kg</td>
<td>23.2 ± 5.1</td>
<td>7.0 ± 1.2</td>
<td>31.0 ± 8</td>
<td>0.89 ± 0.33</td>
</tr>
<tr>
<td>1 nmol/kg</td>
<td>16.2 ± 1.8*</td>
<td>7.7 ± 1.2</td>
<td>26 ± 10</td>
<td>19.4 ± 15.6</td>
</tr>
<tr>
<td>3 nmol/kg</td>
<td>19.4 ± 3.4*</td>
<td>7.0 ± 2.1</td>
<td>33.5 ± 9</td>
<td>33.6 ± 14.9*</td>
</tr>
</tbody>
</table>

* indicates significant difference from vehicle control (p<.05)
Figure Legends

Figure 1: Effect of PYY(3-36) on saline (0.9% NaCl) gastric emptying in rhesus monkeys. Increasing IM doses of PYY(3-36) resulted in increased volume of the original test meal remaining in the stomach at the end of the emptying period. * indicates significant difference from vehicle.

Figure 2: Effect of PYY(3-36) on total 6 hour food intake in rhesus monkeys consuming 1 g pellets. 1 and 3 nmol/kg IM PYY(3-36) resulted in decreased food intake. * indicates significant difference from vehicle.

Figure 3: Hourly cumulative food intake in response to vehicle 1 or 3 nmol/kg PYY(3-36). * indicates significant difference from vehicle.

Figure 4: Effect of chronic 1 nmol/kg PYY(3-36) administration on cumulative daily food intake. Although acute PYY(3-36) significantly reduced intake at this dose, neither the total average intake nor the pattern of ingestion were significantly affected on subsequent days.
Figure 1

**PYY(3-36) and Gastric Emptying**

![Gastric Emptying Graph](image1)

Figure 2

**PYY(3-36) and 6 hr Food Intake**

![Food Intake Graph](image2)
Figure 3

Cumulative Intake

![Cumulative Intake Graph]

- Vehicle
- 1 nmol/kg
- 3 nmol/kg

Figure 4

Feeding Effect of Repeated PYY (3-36)

![Feeding Effect Graph]