Appetite Regulatory Peptides

PYY3-36 as an anti-obesity drug target


Summary

The neuropeptide Y (NPY)/peptide YY (PYY) system has been implicated in the physiology of obesity for several decades. More recently, Batterham et al. 2002 ignited enormous interest in PYY3-36, an endogenous Y2-receptor agonist, as a promising anti-obesity compound. Despite this interest, there have been remarkably few subsequent reports reproducing or extending the initial findings, while at the same time studies finding no anti-obesity effects have surfaced. Out of 41 different rodent studies conducted (in 16 independent labs worldwide), 33 (83%) were unable to reproduce the reported effects and obtained no change or sometimes increased food intake, despite use of the same experimental conditions (i.e. adaptation protocols, routes of drug administration and doses, rodent strains, diets, drug vendors, light cycles, room temperatures). Among studies by authors in the original study, procedural caveats are reported under which positive effects may be obtained. Currently, data speak against a sustained decrease in food intake, body fat, or body weight gain following PYY3-36 administration and make the previously suggested role of the hypothalamic melanocortin system unlikely as is the existence of PYY deficiency in human obesity. We review the studies that are in the public domain which support or challenge PYY3-36 as a potential anti-obesity target.

Keywords: Food intake, obesity, peptide YY, PYY, PYY(3-36), Y-2 receptor.

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Introduction

Peptide YY (PYY, PYY1-36) is a 36 amino acid hormone, first isolated from porcine intestine (1) which, together with pancreatic polypeptide (PP) and neuropeptide Y (NPY), belongs to the pancreatic polypeptide family. These peptides are structurally and biologically similar, but are synthesized and secreted from different sources (2,3). PYY is mainly present in ileum and expressed by large intestine endocrine cells (3–5); it is released postprandially in proportion to the calories ingested (6,7) and acts to inhibit gastric, pancreatic, and intestinal secretion as well as gastrointestinal motility (3,8). Moreover, PYY is also present, although in lower quantity, in the mammalian central nervous system, mainly in the brain stem (9,10). The effects of centrally administered PYY in rodents are well known and include dramatic increases in food intake (mainly carbohydrates), body weight gain, and exploratory activity in rodents (11–15). These effects are putatively believed to be predominantly mediated via the Y1 and Y5 receptors (16–18).

The endogenous peptide form, PYY3-36, is a Y2/Y5 receptor agonist and also produces hyperphagia and obesity syndromes in rats and mice when administered centrally (19–23). However, the orexigenic effects of PYY and NPY, which is equipotent to PYY3-36 in producing hyperphagia, persist in mice with Y1 and Y5 receptor deletions (21,24,25). The Y2 receptor is a putatively presynaptic autoreceptor with inhibitory effects on NPY and PYY release (26,27) and may hence play a role in limiting food intake. Less is known about the effects of peripherally administered PYY or related peptides on ingestive behaviour and energy regulation. The emetic effects of intravenous (i.v.) injection of PYY have been known (28), but, more recently, intraperitoneal (i.p.) administration of PYY3-36 was reported to inhibit food intake in rodents and, after i.v. administration, to decrease appetite in humans (29–31). However, in comparison with the extensive preclinical testing of other appetite-related hormones (e.g. leptin, melanocortin receptor ligands, etc.) prior to human studies, the rapid transition of PYY3-36 from bench to clinic seems unusual, especially given the scarcity of published studies replicating the putative anorectic effects of the peptide in rodents.

Triggered by the exciting findings that peripherally administered PYY3-36 could indeed lower food intake, not only in rodents but also in humans (29), others embarked upon independent investigations. The published anorectic effect of peripherally administered PYY3-36 was largely not replicated or extended in other rodent models (23). Among the few studies reporting anorectic responses, there are inconsistencies and a lack of robust anti-obesity activity with PYY3-36 (31–33). The most promising anti-obesity drug candidates are those that produce their effects reliably in preclinical models and do so robustly across a range of experimental situations (34). Given the number of currently available and regularly discovered new drug targets for obesity, this discrepancy in preclinical research questions the therapeutic significance of PYY3-36. As negative results are rarely accepted for publication in visible scientific journals, the hopes of patients and doctors fighting obesity may be unfounded. In addition, the ability of fellow scientists to make difficult decisions regarding the identity of the molecular targets and/or drug candidates in which to invest may be compromised. The purpose of this review is to evaluate the anti-obesity potential effect of PYY3-36 by presenting all preclinical evidence available on the efficacy of systemic administration of this peptide to reduce food intake and body weight in rodents.

Effect of systemic administration of PYY3-36 on food intake and body weight

Attempts to replicate original study yielding anti-obesity effects of PYY3-36

In order to extend the originally reported anorectic actions of PYY3-36 (29), the present authors and others (23) independently attempted to reproduce the identical original experimental conditions used by Batterham et al. (Table 1). This included use of the exact compound (human PYY3-36), from the same company (Bachem), and with the same catalogue number (H8585) (23). Habituated animals identical in strain, age and gender were administered identical doses of the peptide via the same delivery routes in the same volumes, and under identical light/dark phase, fed/fasted conditions and number of injections (acute and chronic for the same number of days). Food intake and body weights were also measured at the same time points. Despite diligent adherence to the original methods, the initial published results could not be replicated. Most importantly, aside from one study (35) and a second by the authors of the original report (29), no other lab or experiment observed a decrease in body weight or body fat in rodents (Table 1).

Extensions of the original protocol to test for anti-obesity effects of PYY3-36

Whilst replication of published results is important before embarking on drug discovery projects, it was not in the interest of the present authors to challenge the original findings with PYY3-36, but rather to extend them to other obesity-relevant experimental conditions and to further elucidate the peptide’s actions and mediating molecular mechanisms (36). Since then, a few other study groups have also performed such studies (31,33,35,37,38). As itemized in part in Table 1, PYY3-36 was administered to several rodent strains including Wistar, Long Evans, Lister-hooded, and Sprague-Dawleys rats, as well as C57BL/6 J mice,
Table 1: Studies investigating the effects of systemic administration of PYY3-36 in rodents

| Species (male)                              | Route of administration | Injection protocol | PYY3-36 dose                  | Total # of animals (control) | Food intake          | Body weight          | Reference
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>fa/fa rat</td>
<td>pump</td>
<td>Chronic (56 d)</td>
<td>100 μg (25 nmol)/kg/d</td>
<td>20 (14)</td>
<td>No effect</td>
<td>Decreased</td>
<td>(35)</td>
</tr>
<tr>
<td>Lister Hooded rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>3, 30, 100 μg (0.7, 7.4, 25 nmol)/kg</td>
<td>4 x 10 (10)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Ishii et al.</td>
</tr>
<tr>
<td>Long Evans rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>30, 100, 300 μg (7.4, 25, 74 nmol)/kg</td>
<td>32 (8)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Long Evans rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>30, 100, 300 μg (7.4, 25, 74 nmol)/kg</td>
<td>32 (8)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Long Evans rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>30 μg (7.4 nmol)/kg</td>
<td>10 (5)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Long Evans rat</td>
<td>i.p.</td>
<td>Chronic (5 d)</td>
<td>5 μg (1.2 nmol)/100 g</td>
<td>16 (8)</td>
<td>ND</td>
<td>No effect</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>24, 60, 150 μg (5.9, 14.8, 37 nmol)/kg</td>
<td>7 (7)</td>
<td>Decreased</td>
<td>Decreased</td>
<td>(23) – Boggiano (Hagan) et al.</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>3, 10 μg (0.7, 2.5 nmol)/100 g</td>
<td>24 (8)</td>
<td>Decreased</td>
<td>No effect</td>
<td>(23) – Boggiano (Hagan) et al.</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>1.2, 4, 12, 40 μg (0.3, 1, 3, 10 nmol)/kg</td>
<td>5 x 8 (8)</td>
<td>Transient decrease</td>
<td>ND</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>300 μg (74 nmol)/kg</td>
<td>21 (7)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Boggiano (Hagan) et al.</td>
</tr>
<tr>
<td>Sprague-Dawley (diet-induced obese)</td>
<td>i.p.</td>
<td>Acute</td>
<td>3, 10 μg (0.7, 2.5 nmol)/100 g</td>
<td>24 (8)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Boggiano (Hagan) et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>0.3, 3, 10 μg (0.07, 0.7, 2.5 nmol)/100 g</td>
<td>24 (8)</td>
<td>Decreased</td>
<td>Decreased</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>100 μg (25 nmol)/kg</td>
<td>16 (8)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>100 μg (25 nmol)/kg</td>
<td>24 (12)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>100 μg (25 nmol)/kg</td>
<td>24 (8)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>3, 30, 100 μg (0.7, 7.4, 25 nmol)/kg b.i.d.</td>
<td>32 (8)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Schindler et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Acute</td>
<td>100 μg (25 nmol)/kg b.i.d.</td>
<td>24 (6)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Schindler et al.</td>
</tr>
<tr>
<td>Wistar rat (fasted)</td>
<td>i.p.</td>
<td>Acute</td>
<td>5 μg (1.2 nmol)/kg</td>
<td>29 (13)</td>
<td>Increased</td>
<td>ND</td>
<td>(23) – Whitcomb et al.</td>
</tr>
<tr>
<td>Wistar rat (fasted)</td>
<td>i.p.</td>
<td>Acute</td>
<td>5 μg (1.2 nmol)/kg</td>
<td>75 (19)</td>
<td>Increased</td>
<td>ND</td>
<td>(23) – Whitcomb et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Chronic (2 d)</td>
<td>30, 100, 300 μg (7.4, 25, 74 nmol)/kg</td>
<td>32 (8)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Raun et al.</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>i.p.</td>
<td>Chronic (7 d)</td>
<td>5 μg (1.2 nmol)/100 g b.i.d.</td>
<td>8-12</td>
<td>Decreased</td>
<td>Decreased</td>
<td>(23) – Raun et al.</td>
</tr>
<tr>
<td>Agouti pre and postobese mice</td>
<td>i.p.</td>
<td>Acute</td>
<td>80 μg (20 nmol)/kg</td>
<td>5-6 (5-6)</td>
<td>Decreased</td>
<td>Decreased</td>
<td>(23) – Raun et al.</td>
</tr>
<tr>
<td>C57BL6 J mice</td>
<td>i.p.</td>
<td>Acute</td>
<td>0.3, 3, 10 μg (0.07, 0.7, 2.5 nmol)/100 g</td>
<td>4-5 (10)</td>
<td>Decreased</td>
<td>NP</td>
<td>(31)</td>
</tr>
<tr>
<td>C57BL6–129sv mice</td>
<td>i.p.</td>
<td>Acute</td>
<td>0.3, 3, 10 μg (0.07, 0.7, 2.5 nmol)/100 g</td>
<td>5 (5)</td>
<td>Decreased</td>
<td>NP</td>
<td>(29)</td>
</tr>
<tr>
<td>C57BL6 mouse</td>
<td>s.c.</td>
<td>Acute</td>
<td>4.2, 125 μg (1, 3 nmol)/kg</td>
<td>3 x 6 (6)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Moran et al.</td>
</tr>
<tr>
<td>C57BL6 mouse</td>
<td>s.c.</td>
<td>Acute</td>
<td>4.2 μg (1 nmol)/kg</td>
<td>4 x 6 (12)</td>
<td>No effect</td>
<td>ND</td>
<td>(23) – Moran et al.</td>
</tr>
<tr>
<td>C57BL6 mouse</td>
<td>s.c.</td>
<td>Chronic (7 d)</td>
<td>1 mg (247 nmol)/kg</td>
<td>17 (9)</td>
<td>Transient decrease</td>
<td>No effect</td>
<td>(23) – Heiman et al.</td>
</tr>
</tbody>
</table>
Table 1  Studies investigating the effects of systemic administration of PYY3-36 in rodents (Continued)

<table>
<thead>
<tr>
<th>Species (male)</th>
<th>Route of administration</th>
<th>Injection protocol</th>
<th>PYY3-36 dose</th>
<th>Total # of animals (control)</th>
<th>Food intake</th>
<th>Body weight</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 mice on high-fat diet</td>
<td>pump</td>
<td>Chronic (28 d)</td>
<td>30, 100, 300, 1000 μg</td>
<td>98 (42)</td>
<td>Decreased**</td>
<td>Decreased**</td>
<td>(35)</td>
</tr>
<tr>
<td>I29/J mice</td>
<td>i.p.</td>
<td>Acute</td>
<td>10 μg (2.5 nmol)/100 g</td>
<td>8 (8)</td>
<td>No effect</td>
<td>No effect</td>
<td>(32)</td>
</tr>
<tr>
<td>NIH/Swiss mice (female)</td>
<td>i.p.</td>
<td>Acute</td>
<td>0.1, 1, 3, 10, 30, 100, 300, 1000 μg (0.025, 0.25, 0.7, 2.5, 7.4, 25, 74, 247 nmol)/kg</td>
<td>192 (90)</td>
<td>Decrease</td>
<td>Decrease**</td>
<td>(35)</td>
</tr>
<tr>
<td>NMRI mouse</td>
<td>i.p.</td>
<td>Acute</td>
<td>0.25, 0.7, 2.5, 7.4, 25, 74, 247 nmol/kg</td>
<td>18 (6)</td>
<td>No effect</td>
<td>No effect</td>
<td>(23) – Tschöp et al.</td>
</tr>
<tr>
<td>NZO mouse</td>
<td>i.p.</td>
<td>Chronic (10 d)</td>
<td>100 μg (25 nmol)/kg b.i.d.</td>
<td>14 (7)</td>
<td>Increase (−20%)</td>
<td>Increase (−5%)</td>
<td>(23) – Tschöp et al.</td>
</tr>
<tr>
<td>ob/ob mouse</td>
<td>s.c.</td>
<td>Chronic (7 d)</td>
<td>1 mg (247 nmol)/kg</td>
<td>17 (9)</td>
<td>Transient decrease</td>
<td>Transient decrease</td>
<td>(23) – Heiman et al.</td>
</tr>
<tr>
<td>ob/ob mice (female)</td>
<td>pump</td>
<td>Chronic (28 d)</td>
<td>100, 300, 1000 μg (25, 74, 247 nmol)/kg/d</td>
<td>32 (8)</td>
<td>No effect</td>
<td>Decreased**</td>
<td>Decreased**</td>
</tr>
</tbody>
</table>

i.p., intraperitoneal administration; s.c., subcutaneous administration; i.v., intravenous administration; b.i.d., twice a day; ND, not determined; NP, not provided in publication; MW of PYY3-36 is 4050.0 D.

*For studies with multiple independently run studies, the name preceding the reference number is the lab PI(s).

† In non-diabetic fa/fa rats; diabetic fa/fa rats had increase in body weight with PYY3-36.

‡ No effect on total 1-h intake but a significant increase, −25–50% in duration of feeding in the first 10 min of the test.

§ Wistar rats were also infused with 10 μg (2.5 nmol)/5 μL PYY3-36 (i.c.v) and increased food intake −200%; 3, 4 and 8 μg (2 nmol)/3 μL i.c.v. increased food intake −300%. Long Evans rats also increased intake with 4.2 μg (1 nmol) PYY3-36 i.c.v. by −60%. These studies confirm the bioactive viability of the PYY3-36 sample used in peripheral injections.

‖ Only the 60 μg (14.8 nmol) dose decreased intake of liquid Ensure and only for 3 h with the suppression appearing to be inversely related to dose. Lesions of the area postrema in a separate group of rats (n = 7) were found to increase the magnitude and duration of PYY3-36-induced anorectic effects.

¶ Transient decrease was −70% only at 30 min with 4.2 μg (1 nmol)/kg. Other dose and time points were without effect.

‖ Rats were maintained on standard chow, or a high-fat diet (HFD), or were 50% chow-restricted. The PYY3-36-induced decrease in 24-h intake was slight, −10% for chow- and HFD-fed; no effect in 50% chow-restricted rats.

‡‡ Mice were 24 h fasted; intake of all mice was decreased at 2 h and in only post-obese mice at 24 h.

§§ Mice were 16 h fasted; a dose of 0.3 μg (0.07 nmol) actually increased food intake; decreased intake with 3 and 10 μg (0.7 and 2.5 nmol) was reported as statistically significant but represented an exceedingly small amount of 4-h food intake reduction (within a range of less than one gram).

¶¶ Control rats were Y2-receptor null mice whose intake was not affected by PYY3-36.

|| −30% decrease only in the first 3 d.

∗ Food intake was measured on a ‘per cage’, not per mouse basis (4–5 mice per cage).

** Food intake was reduced only with the highest dose (1 mg or 247 nmol/kg/d); body weight was reduced with a minimum of 300 μg (74 nmol/kg/d).

*** Suppression of food intake occurred at 6 and 24 h only in 24-h fasted, but not freely fed mice.

**** Effects were obtained only with doses 10 μg (2.5 nmol)/kg, highest reduction −45% with 100 μg (25 nmol/kg) dose, total decreases in intake constituted 0.8–2 g decrease/1 h.

***** −2% decrease on first day only.

****** 0 to −4% change from baseline weight with PYY3-36 vs. 6% increase in baseline weight with saline.

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NMRI mice, New Zealand obese mice (23), and non-fasted 129/J mice (32). Various methods of drug delivery were tested including i.p., i.v., subcutaneous (s.c.), once or twice a day, and continuously via osmotic pump. These treatments were administered prior to both the dark and light phase, and food intake was measured at 30 min and 1, 2, 3, 4, 6, 8, and 24 h in acute experiments and following several days in chronic experiments. In 24 of 37 studies, no difference in food intake was found. Interestingly, in as many as four of these studies, a statistically significant increase in food intake was observed. Only 10 separate studies found a decrease in food intake, but, if these, nine reported either the need for very high dosing (35), that animals be fasted (32,38), a paradoxical inverse dose-response (33), or a weaker (31,37) and more transient (23) suppression of intake despite chronic dosing, than originally reported (29). Similarly disappointing was the overwhelming lack of suppression in body weight obtained when several mouse (C57BL/6J, NMRI, New Zealand obese, ob/ob) and rat (Wistar, Lister-hooded, Sprague-Dawley, Long Evans) strains were tested. In these studies, animals were administered 3, 5, 50 and 100 μg (0.7, 1.2, 12.3 and 23 nmol/kg), 5 μg (1.2 nmol/100 g), 1 mg (247 nmol) kg/d, 5 μg (1.2 nmol)/100 g/d; i.p., i.v., s.c., once or twice per day, or continuous infusion prior to both the dark and light phase. Body weight was measured over 2, 5, 7, 8 or 10 d. In 11 of our 14 studies, no change in body weight was observed in comparison with vehicle-treated controls. In one of the experiments, a transient decrease of body weight was observed on the first day of treatment, but not at any other time point after continuous administration with minipump (Table 1). As occurred with food intake, there was a significant increase of body weight in two of the studies. Body composition was monitored daily in two of these mouse experiments using a novel quantitative magnetic resonance analyser. No change in lean mass was found in either study, while fat mass actually increased in one study and did not change in the other. Another concern with the few experiments reporting weight loss is the frequent use of very young animals, as young as 28 d (mice) and 52 d (rats) (29,35). The reported weight losses, compared with saline-treated control rodents, may therefore represent a decrease in lean mass.

In the original Batterham et al. 2002 study (29), rapidly growing rats gained 60 g in 7 d (about 25% of their body weight), a gain most likely of muscle and bone mass, not fat mass. Pharmacological blockade of such weight gain likely represents changes in lean rather than in fat mass.

In the one subsequent study that found decreased body weight, high doses of PYY3-36 (300–1000 μg or 74–247 nmol/kg/d) were needed, and these were chronically infused via minipump for extended durations (28–56 d) (35). Interestingly, in this same study PYY3-36 accelerated body weight gain of obese and diabetic fa/fa rats.

Some of the present authors were the first to test and report the effects of PYY3-36 in obese models, observing no anorectic or weight loss effects in diet-induced obese Sprague Dawley rats, New Zealand obese mice or ob/ob mice (23). In an effort to extend the reported effects of PYY3-36 on food intake and energy balance, feeding behaviour, spontaneous locomotor activity, energy expenditure (via indirect calorimetry) and other related behaviours, such as rearing, grooming, resting, sniffing and taste aversion, were also measured (23,39). Contrary to expectations for catabolic actions, there was a significant (though short-lasting) increase in feeding behaviour, along with indices of decreased motor activity (Table 2), overall energy expenditure, and caloric content of the faeces following treatment with PYY3-36 compared with saline.

Discrepancies within studies reporting anti-obesity effects of PYY3-36

There are a number of discrepancies among the few studies suggesting that PYY3-36 decreases food intake. Specifically, the reported duration of PYY3-36 anorexia was much less persistent than originally reported (31,32,38). There was also a smaller magnitude of these effects, which were only observed after pre-fasting, as well as the finding of no effect at all in freely fed animals (32). An anti-obesity drug should be robust across fed and fasted states and should remain potent for extended periods of time. In one study (31), food intake suppression did not last beyond 12 h, and the suppression was in the range of only 0.2 g in groups of four to six mice. Measuring such small differences would require very precise instrumentation, but the mice were simply presented with standard laboratory chow pellets in Petri dishes that where manually weighed. Several studies trying to replicate the reported anorectic effects of PYY3-36 used state-of-the-art computerized online monitoring equipment to measure food intake. One investigation (Table 2) that included video recording and treatment-blind ethological analyses revealed that PYY3-36 dose-dependently reduced the latency and increased the frequency and duration of eating in tests with palatable mash (39). Of those that did find a hypophagic effect with PYY3-36, a surprising inverse dose effect trend was noted (33), while others reported previously effective doses to be ineffective in reducing food intake and body weight (31). A study by Nordheim and colleagues investigated cardiovascular effects in rats after PYY3-36 treatment and observed ‘slight’ reductions in food intake (37), but ones that were not comparable with those reported by Batterham et al. However, in mice, any feeding-suppression by PYY3-36 was more likely to occur when they were hungry (16- and 24-h fasted) prior to injection (Table 1) (32). This would suggest that the peptide has significant limitations in its actions, interacting only with physiological signals specifi-
Table 2  Effects of PYY3-36 on feeding latency, locomotor, and other behaviours of rodents

<table>
<thead>
<tr>
<th>Behavioural measure</th>
<th>Saline</th>
<th>3 µg/kg</th>
<th>30 µg/kg</th>
<th>100 µg/kg</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eat latency seconds</td>
<td>38.70 ± 8.15</td>
<td>18.48 ± 4.72</td>
<td>15.90 ± 2.19</td>
<td>12.46 ± 1.86*</td>
<td>F(3,21) = 6.45, P = 0.025</td>
</tr>
<tr>
<td>Eat duration seconds</td>
<td>744.45 ± 62.20</td>
<td>758.40 ± 57.71</td>
<td>738.59 ± 55.14</td>
<td>804.54 ± 47.99</td>
<td>F(3,27) = 1.27, NS</td>
</tr>
<tr>
<td>Eat frequency</td>
<td>51.20 ± 8.24</td>
<td>53.00 ± 9.02</td>
<td>45.40 ± 6.88</td>
<td>51.10 ± 7.35</td>
<td>F(3,27) = 1.60, NS</td>
</tr>
<tr>
<td>Drink duration seconds</td>
<td>0.11 ± 0.35</td>
<td>0.00 ± 0.00</td>
<td>0.73 ± 1.53</td>
<td>0.43 ± 1.14</td>
<td>F(3,27) = 1.20, NS</td>
</tr>
<tr>
<td>Drink frequency</td>
<td>0.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>0.20 ± 0.13</td>
<td>0.20 ± 0.13</td>
<td>F(3,27) = 1.00, NS</td>
</tr>
<tr>
<td>Locomotion duration seconds</td>
<td>912.64 ± 65.85</td>
<td>906.33 ± 84.74</td>
<td>892.17 ± 91.62</td>
<td>799.59 ± 96.05</td>
<td>F(3,27) = 1.45, NS</td>
</tr>
<tr>
<td>Locomotion frequency</td>
<td>223.60 ± 14.41</td>
<td>205.50 ± 19.08</td>
<td>200.70 ± 11.73</td>
<td>203.80 ± 17.01</td>
<td>F(3,27) = 1.25, NS</td>
</tr>
<tr>
<td>Rear duration seconds</td>
<td>653.92 ± 51.54</td>
<td>554.16 ± 66.46</td>
<td>470.91 ± 52.88**</td>
<td>424.85 ± 73.22**</td>
<td>F(3,27) = 12.39, P &lt; 0.001</td>
</tr>
<tr>
<td>Rear frequency</td>
<td>156.00 ± 10.37</td>
<td>138.20 ± 12.60</td>
<td>117.70 ± 10.63**</td>
<td>109.30 ± 13.04**</td>
<td>F(3,27) = 8.34, P &lt; 0.001</td>
</tr>
<tr>
<td>Groom duration seconds</td>
<td>234.46 ± 20.11</td>
<td>314.37 ± 52.30</td>
<td>274.81 ± 36.19</td>
<td>265.09 ± 32.92</td>
<td>F(3,27) = 0.85, NS</td>
</tr>
<tr>
<td>Groom frequency</td>
<td>23.60 ± 2.82</td>
<td>23.30 ± 2.72</td>
<td>23.00 ± 2.26</td>
<td>23.50 ± 2.20</td>
<td>F(3,27) = 0.01, NS</td>
</tr>
<tr>
<td>Rest duration seconds</td>
<td>216.94 ± 84.87</td>
<td>340.01 ± 142.31</td>
<td>241.39 ± 94.15</td>
<td>209.77 ± 102.85</td>
<td>F(3,27) = 0.81, NS</td>
</tr>
<tr>
<td>Rest frequency</td>
<td>11.30 ± 3.84</td>
<td>10.60 ± 3.80</td>
<td>13.50 ± 4.02</td>
<td>10.60 ± 3.18</td>
<td>F(3,27) = 0.47, NS</td>
</tr>
<tr>
<td>Sniff duration seconds</td>
<td>771.43 ± 88.76</td>
<td>696.78 ± 62.52</td>
<td>661.41 ± 89.40</td>
<td>104.15 ± 137.85*</td>
<td>F(3,27) = 6.12, P &lt; 0.003</td>
</tr>
<tr>
<td>Sniff frequency</td>
<td>127.40 ± 12.51</td>
<td>110.30 ± 13.56</td>
<td>126.90 ± 13.45</td>
<td>140.20 ± 16.20</td>
<td>F(3,27) = 3.32, P &lt; 0.035</td>
</tr>
</tbody>
</table>

Doses of 3, 30, 100 µg are equivalent to 0.7, 7.4, and 25 nmol respectively; *P < 0.05, **P < 0.03 vs. saline; measures were during a 1-h-test with palatable mash in adult male Lister-hooded rats initially habituated to the test arena and test food for 1 h daily for 5 d prior to the experiment, and with additional basal sessions run weekly throughout the study. Human PYY3-36 (Bachem) was used and given in a complete repeated measures design (n = 10). Videotapes were scored blind by two highly trained observers (intra-rater and inter-rater reliability ≥ 0.8 [Ishii et al. (23,39)]. NS, not significant.

Factors potentially explaining the discrepant findings with PYY3-36 on food intake and body weight

Species-specificity and bioactivity of PYY3-36

Those unable to replicate the anorectic effects of PYY3-36 considered the importance of species-specificity in compounds used in in vivo pharmacology. Although Batterham et al. 2002 did not specify the species form of PYY3-36 used in their rodent studies, they cited Bachem as the commercial source and, at that time, Bachem supplied only human PYY3-36. For this reason, human PYY3-36 was used in subsequent attempts to reproduce the first reported anorectic effects of the peptide. Since then, the original authors have confirmed their use of human PYY3-36 (personal emails and communication at the Endocrine Society Meeting, San Francisco, June 2002). Regardless of the exact compound used, it has routinely failed to suppress food intake. Some investigators also tested rat PYY3-36 [synthesized by the UCLA Peptide Synthesis Laboratory, a generous gift from Dr J. Reeves (23)] and, as with human PYY3-36, no decrease in food intake was observed.

Companies were also solicited to synthesize PYY3-36 species-independent compounds, and these were tested separately in multiple mice and rat bioassays for relevant receptor sub-types, namely Y1, Y2 and Y5. In addition, some investigators have used pure (tested by HPLC and GCMS) PYY3-36. Bioactivity was confirmed for all of these PYY3-36 compounds, in which they either increased food intake following intracerebroventricular (i.c.v) administration or caused delayed gastric emptying following peripheral administration, both well established effects of PYY3-36 (8,11,12,40–42). Delayed gastric emptying is believed to be mediated via Y2 receptors (43), while Y1 and Y5 receptors have been implicated in the centrally induced hyperphagic response (16–18). Together, these measures attest to the functional integrity of the compounds tested (Fig. 1A–D), and indicate that, independent of the source (e.g. Bachem, Eli Lilly, Polypeptides, Neosystems, Tocris, Peptides & Elephants) and after confirmation of their central and systemic bioactivity in rodents, PYY3-36 did not decrease food intake in mice and rats (Figs 2A–D and 3A–D respectively) or decrease body weight (Fig. 4). As such, concerns about diverging sub-type specificity and bioactivity seem unlikely to be a potential explanation for the discrepant results. Another aspect to consider is the pharmacokinetic properties of PYY3-36. Little is known regarding the relevant plasma levels of
PYY3-36 achieved after i.p. or i.v. administration. A high initial \( C_{\text{max}} \) of the peptide, for example, may exert anorectic effects that would explain why after i.v. application some investigators have found transient anorectic effects. I.p. injection mimics the more natural (postprandial) condition of more slowly rising plasma PYY3-36 levels. Additional pharmacokinetic data are needed to evaluate this as a possible explanation for the discrepant results.

Are non-PYY3-36-responding animals stressed?

The possibility has been suggested that insufficient habituation procedures can preclude the detection of an anorectic response to PYY3-36 (44). The merit of this as a putative reason for discrepant PYY3-36 seems unlikely on several levels. First, in the original publication (29), Batterham et al. state that their rats were accustomed to i.p. injections of 0.5 mL of saline for 2 d prior to the study.

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**Figure 1** (A) Quality and identity check of 38.9 µg (9.6 nmol)/mL PYY3-36 used for in vivo studies. PYY3-36 (Bachem) was dissolved in phosphate-buffered saline, stored at room temperature and analysed 3 d later. Sample was analysed by HPLC and detected by UV at 214 nm. Peptide purity was estimated to ~94% and the concentration to 38.6 µg (9.5 nmol)/mL. Insert: The identity of the sample was analysed by LC-MS to yield a MH²⁺ ion with \( m/z \) of 1350.8 corresponding to a MW of 4049.4; calculated MW 4049.6 (Madsen & Kjellander (23)). (B) Comparison of solutions of PYY3-36 (Bachem lot no. 52228) prepared fresh (\( \bullet \)) or the remainder from in vivo studies (\( \bigtriangledown \)) analysed in human NPY Y2 receptor binding assays using \(^{125}\)I-PYY (Perkin Elmer) as radioligand. Both solutions gave essentially the same biological activity, 0.8 µg (0.2 nmol) vs. 1.6 µg (0.4 nmol), \( P < 0.05, n = 2 \). Peptide was reconstituted in phosphate buffered saline and analysed immediately or following 3 d at room temperature. Assays were performed using recombinant human NPY Y2 receptors expressed in and extracted from BHK tk-ts13 cells. 1.5 µg membrane protein was mixed with 0.2 µg (50 pM) \(^{125}\)I-PYY and varying concentrations of PYY3-36 in buffer (25 mM HEPES, 2.5 mM CaCl\(_2\), 1.2 mM MgCl\(_2\), 0.1% BSA, pH 7.4). Radioligand binding was determined using WGA-coated SPA beads and TopCount NTX (Stidsen, Wulff, & Schiødt (23)). (C) Stimulation of food intake after i.c.v. administration of 1 µg (0.25 nmol)/kg PYY3-36 (Saxon) or 0.5 µg (0.12 nmol)/kg NPY (Polypeptide Laboratories) in the lateral ventricle of fed, female Wistar rats (230–250 g, 12/12 h light cycle), \( P < 0.05, n = 10 \). Compounds had been administered 2 h before the beginning of the dark phase. Rats had been daily trained for handling for 2 weeks (Schoelch, Arndt, Rudolph, Mark & Schindler (23)). (D) Gastric emptying slowing by 12 µg (3 nmol) i.p. PYY3-36 (Bachem) in \( n = 8 \) male Sprague Dawley rats adapted to a daily intragastric tubing procedure for one week prior to start of testing; *\( P < 0.05 \) (Kinzig, Behles, Scott & Moran (23)).
Later, Halatchev et al. (31) specifically compared injection-acclimated with non-injection-acclimated mice and reported anorectic effects of PYY3-36 only in injection-acclimated mice. More recently, researchers at Amylin Pharmaceuticals testing PYY3-36 in rodents stated that their animals were acclimated for one week, but did not describe what this involved, short of acclimation to the light/dark cycle (35). In direct contrast, studies failing to find anorectic effects included standard protocols for thorough habituation to chronic and repeated handling, mock injections and food intake measures (23). The animals were extensively adapted to the experimental environment and showed no signs of stress before experimentation. More important however, is the issue of acclimatization to drug delivery in humans. If a drug is so sensitively contingent on several first experiences with an inactive agent, it will require a most unusual protocol for patients.

Secondly, if stress of injection affects appetite, it should do so equally, regardless of the anorectic agents that are administered in the same manner. In this context, some experiments included acute systemic administration of cholecystokinin (CCK), MT-II (melanocortin 3/4-receptor agonist), and/or naloxone (opioid receptor antagonist) as positive controls to PYY3-36 administration. While the former agents effectively suppressed appetite under the exact same experimental conditions, PYY3-36 did not.

Thirdly, although any non-specific effects of stress on appetite and body weight gain should have been evident in saline-treated controls, these animals did not lose weight or decrease their food intake (Figs 2–4). Fourthly, it is curious that, when PYY3-36 has been reported to decrease

Figure 2 (A) Intraperitoneal (i.p.) administration of 100 µg (25 nmol)/kg PYY3-36 (Peptides & Elephants), twice per day for 10 d in male NMRI mice increased food consumption ($P<0.05$, $n=7$). (B) i.p. administration of 100 µg (25 nmol)/kg PYY3-36 (Bachem), twice per day for 8 d in male New Zealand obese (NZO) mice failed to decrease food consumption; $n=4–6$ [Castañeda, Thöne-Reineke, Ortmann, Klaus, Birringer, Kreuzer, Jonst & Tschöp (23)]. (C) i.p. PYY3-36 (Bachem) on cumulative food intake in male C57BL/6 J mice did not decrease food intake; $n=6$ [Kinzig, Behles, Scott, & Moran (23)]. (D) Continuous subcutaneous (s.c) injections of 1 mg (247 nmol)/kg/d PYY3-36 (Lilly) for 7 d in male C57BL/6 mice decreased food consumption during the first 3 d of treatment; $P<0.05$; $n=8–9$, but the effect was not sustained thereafter [Craney, Smiley, Flora & Heiman (23)].
food intake, it apparently does so exclusively, or at least more effectively, in mice that are pre-fasted for at least 24 h (Table 1 footnotes). Based on the much faster metabolism of rodents, this would translate into a period of complete fasting for about one week for humans before PYY3-36 would have an effect on the size of a following meal. Furthermore, fasting is known to increase plasma corticosteroid levels in rodents, a well-known stress response (45). Hence, if PYY3-36 effects are inhibited by stress, any anorectic effects of the peptide should be more readily detected in fed, not fasted, animals. The opposite, however, seems to be the case. Moreover, the possibility that one must follow a strict injection habituation protocol in order to observe PYY3-36-induced hypophagia (31) is also challenged by the findings of Nordeim et al. (37). They detected slight 24 h reductions in intake with PYY3-36 in rats that had only one week of acclimation to the environment (no mock saline injections) prior to extensive surgical procedures (abdominal incision, intestinal retraction, arterial re-sectioning and telemetry transmitter transplantation) and introduction to unfamiliar dietary regimens (some that included restriction). Clearly, these animals were not without stress.

In summary, it seems highly unlikely that stress arising from inadequate habitation procedures can explain the discrepant results with PYY3-36, given that so many studies have systematically controlled for such effects. It is also unrealistic to expect all laboratories worldwide to follow identical protocols when testing any anti-obesity drug. Rather, drugs with expected effects on rodents’ ingestive...
behaviour should decrease food intake robustly across diverse environments. More importantly, a potential anti-obesity drug should work robustly against similarly varying circumstances in humans. Stress is certainly a relevant condition in human obesity (46) and can, under certain circumstances, even be a salient stimulus for overeating (47,48). Hence, if stress were playing a role in precluding PYY3-36-induced hypophagia, even in the absence of evidence for such a causal relationship, then long-term treatment of obesity with this peptide poses quite a challenge, a concern recently echoed by O’Rahilly and colleagues (44).

Statistical issues: power and significance

Can the discrepancy in responses with PYY3-36 be explained simply by reference to stochastic factors? Although a few of the present reviewers’ studies produced results in the direction predicted by Batterham et al. (29), their number, relative to the total number of these investigator’s independent experiments conducted (assuming all tests were done at a 2-tailed 0.05 level), was not significantly greater than expected, when referred to the binomial distribution (for food intake 2 of 41 yields \( P = 0.273 \); for body weight 1 of 11 yields \( P = 0.243 \)). In contrast, the number in the unexpected direction was significantly greater than expected by chance, given the number of hypotheses tested (for food intake 4 of 41 yields \( P = 0.019 \); for body weight 2 of 11 yields \( P = 0.030 \)). Might one conjecture that failure to reproduce an anorectic effect with PYY3-36 is the result of insufficient power or type II errors? In their original publication, Batterham et al. (29) stated that PYY3-36 that was administered i.p. twice daily for 7 d reduced cumulative food intake (7-d cumulative food intake: PYY3-36, 187.6 ± 2.7 g; saline, 206.8 ± 2.3 g; \( n = 8 \) per group, \( P < 0.0001 \)) and decreased body weight.
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PYY3-36 as anti-obesity drug

The role of DPP-IV in the conversion of PYY1-36 to PYY3-36

It has recently been reported that mice deficient for dipeptidyl peptidase (DPP-IV), the enzyme that cleaves PYY1-36 into PYY3-36, which should suffer from complete PYY3-36 deficiency, are not obese, but are protected against diet-induced obesity and hyperinsulinemia. These PYY3-36 deficient mice also show reduced food intake and increased energy expenditure. Although this finding (50) may reflect not only lack of PYY3-36, but also an increased presence of active glucagon-like peptide 1 (GLP-1) and glucose-dependent insulino-metabolite polypeptide (GIP), this observation is in strong contrast with an essential role for PYY3-36 as a physiological satiety factor. However, the results reported on DPP-IV knockout mice are consistent with observations showing that chronic administration of PYY3-36 in NMRI mice increase food intake and body weight (23) and with why acute treatment with PYY3-36 attenuates activity (Table 2).

Hypothalamic receptor sub-types activated by PYY3-36

PYY3-36 is a potent agonist of Y2 and Y5 receptors (22,51,52). Some of the present authors conducted their own in vitro binding, i.e. using SMS-KAN-, HEC1b-Hy5- or SK-N-MC-cells to perform classic ligand-receptor binding studies (UCLA Peptide Synthesis Laboratory). They observed equally strong binding of PYY3-36 and PYY1-36 at Y2-receptors, moderate binding at Y5-receptors, and substantially weaker binding of PYY3-36 as compared with PYY1-36 at Y1-receptors (23). Both Y2 and Y5 receptor types are present in the rodent brain in specific loci with varying density (3). While Y5 receptors are believed to be responsible, at least in part, for the orexigenic effects of NPY and PYY in the CNS, Y2 are putative presynaptic autoreceptors that may mediate negative autocrine feedback of NPY onto NPY expressing neurones (26,27). Batterham and colleagues put forward the very elegant hypothesis that PYY3-36, which easily crosses the blood brain barrier (53), acts predominantly on Y2 receptors in the arcuate nucleus of the hypothalamus to reduce food intake (29). Y2 ligands administered centrally may suppress food intake in rodents (54), although evidence on action of such compounds still is more than scarce, and mice with complete Y2-receptor deletion are reported to have increased body weight and food intake (27). Y5 receptors are more concentrated in the area postrema (AP) than in the hypothalamus (53), and lesions of the AP have recently been shown to transiently enhance possible anorectic potency of PYY3-36 (33). These biological observations are principally consistent with the hypothesis that PYY3-36 may be inhibiting food intake via more selective stimulation of Y2-receptors than PYY1-36.

Molecular analysis of PYY3-36: possible explanations for inconsistent responses

A more productive and valuable strategy in speculating on the reasons for lack of PYY3-36 in so many investigators’ hands is to consider the peptide’s molecular and physiological properties.

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to changes in metabolic parameters after centrally administered PYY3-36 than after centrally administered NPY (27). In actuality, the exact role of Y2 receptors in feeding and energy regulation remains unknown. Deletion of the Y2-receptor targeted mouse mutagenesis resulted in transiently increased food intake but decreased body weight (57). The investigators of this study also found that complete germ-line Y2 deletion resulted in decreased body weight and decreased food intake (57). Although Batterham and colleagues (29) concluded that Y2 receptors mediate PYY3-36-induced hypophagia, based on its lack of effect in Y2 deficient mice (29), the strong inconsistencies throughout all published wildtype rodent studies challenge the specificity of the lack of response in Y2-knock-out mice and therefore the above mentioned conclusion. It also seems relevant to note that mice fasted for 48 h do not exhibit any change in arcuate nucleus Y2 receptor expression (58).

Other molecular mechanisms that could mediate PYY3-36 induced satiety

Peripheral actions of PYY3-36 may also play a role in the transient reductions in food intake observed in some laboratories. Decreased gut motility and faecal output is mediated via Y2 receptor activation (3), and it is well known that PYY slows gastric emptying and increases intestinal transit time. For example, Y2 agonists and PYY applied in the dorsal vagal complex suppress thyrotropin-releasing hormone (TRH) stimulated gastric motility (26). A temporary decrease in food intake may therefore also be the result of visceral illness, because it has been reported that PYY slows gastric emptying and increases intestinal transit time. For example, Y2 agonists and PYY applied in the dorsal vagal complex suppress thyrotropin-releasing hormone (TRH) stimulated gastric motility (26). A temporary decrease in food intake may therefore also be the result of visceral illness, because it has been reported that PYY slows gastric emptying and increases intestinal transit time. For example, Y2 agonists and PYY applied in the dorsal vagal complex suppress thyrotropin-releasing hormone (TRH) stimulated gastric motility (26). A temporary decrease in food intake may therefore also be the result of visceral illness, because it has been reported that PYY slows gastric emptying and increases intestinal transit time. For example, Y2 agonists and PYY applied in the dorsal vagal complex suppress thyrotropin-releasing hormone (TRH) stimulated gastric motility (26).

However, how precisely PYY3-36 interacts with ghrelin is unknown at present and a possible role for circulating human ghrelin in food intake regulation is still controversial.

Little is also known about the exact central mechanisms by which PYY3-36 might induce satiety. Impressive and elaborate electrophysiology studies reported by Batterham and colleagues strongly suggested activation of proopiomelanocortin (POMC) neurones as a central mechanism for PYY-induced satiety (29). However, more recently, food intake and body weight in POMC-deficient mice have been found to be unaffected by peripheral administration of PYY3-36 (62). Consistent with these findings, PYY3-36 also has no effect on food intake in mice deficient for the melanocortin-4 receptor (31), which is known to ultimately mediate changes in POMC expression after activation by POMC derived α-MSH. The discrepancies in studies implicating a role for the melanocortin system may be the result of species-specific differences between rats (in which the electrophysiology studies were performed) and mice (in which the pharmacological studies after targeted mutagenesis were performed). However, such differences are not typical, and there is no other evidence to support such an explanation. Coll et al. (2004) offer the possibility that the anorectic cocaine- and amphetamine-regulated transcript (CART) and the neurotransmitter gamma-aminobutyric acid (GABA) may be candidates to mediate PYY3-36-induced effects. These substances are known to be partially co-expressed with and released by POMC neurones respectively (44); however, there is no known colocalization between Y2-receptors, GABA and CART. Thus far, the most consistent central changes evoked by PYY3-36 administration seem to be reductions in hypothalamic NPY expression, supporting a putative role of Y2 activity in effects of PYY3-36 (29,32). Most recently, however, R. Cone and colleagues concluded, via an elaborate series of studies, that PYY3-36 causes visceral illness and conditioned taste aversion as well as c-fos expression in the area postrema and nucleus tractus solitarius, brain regions known to mediate these responses (63). These findings may explain why some groups have observed small transient effects on food intake without body weight effects following systemic PYY3-36. This is an effect that is very different from the anorectic and catabolic effects of melanocortin agonist compounds such as MT-II.

Some have questioned whether it is important at all to understand the molecular mechanisms mediating PYY3-36 action, if it successfully treats obesity (44). At one level, if a compound is clinically efficacious (and of course safe), understanding its physiology might be secondary. However, to date, no peer-reviewed report has described actual weight loss following administration of PYY3-36 in humans.

Comparison of PYY3-36 with the status of other satiety agents as anti-obesity targets

If PYY3-36 is truly a powerful satiety agent with the potential to treat obesity, conditions under which it successfully works should not have to be defined down to the most meticulous details. Before it can reasonably be chosen as a clinical candidate, there should be repeatable and robust dose-dependent effects across multiple animal models in multiple independent laboratories. A notable difference between the bench-to-clinic evolution of PYY3-36 and other anti-obesity candidates is the paucity of such published preclinical investigations, which typically precedes a human drug target. For example, 2 years after the first
reports of leptin-induced satiety and ghrelin-induced food intake, numerous reports (over 500 for leptin and 200 for ghrelin) from many independent laboratories had supported and extended the original findings in rodents. In contrast, since the initial report on PYY3-36 (29), only six supporting articles have been published, and the majority of these document various experimental contingencies and caveats (e.g. dependence on unique habituation protocol, transience of effects, lack of effect in fed animals, absence of clear dose-dependency, no effect on body weight, dependence on extensive pre-fasting).

**Human studies with PYY3-36**

The first report on PYY plasma levels (total PYY; immunoassays to specifically quantify PYY3-36 are not yet available) suggested that PYY is low in obese individuals, that a PYY-deficiency may be contributing to their obesity, and, hence, that PYY replacement therapy might be useful (30). However, the same investigators recently reported that, using the same assay, PYY levels in obese individuals were either normal or slightly increased (64). The same was recently reported by some of the present authors in a much larger population of lean, obese and morbidly obese individuals (65).

Based on the first very promising and enthusiastic report of PYY3-36 as an anti-obesity agent, several research groups have explored its effects in humans. In a first (double-blind crossover) study, described alongside the original report of anorectic effects in rodents (29), 12 fasting non-obese young adults, infused with 3.2 ng (0.8 pmol)/kg PYY3-36 over 90 min, ate 33% less food over 24 h than when infused with saline. In the second study, 12 fasted obese and non-obese young adults were i.v. infused with 8 µg (2 nmol)/m² body surface area PYY3-36 over 90 min. Compared with saline infusion, obese and non-obese subjects reduced their 24-h intake by 26% and 34% respectively, with suppression occurring within and not beyond the first 12 h post-infusion (30). These studies were criticized in regard to the reported 100% efficacy in all treated subjects with only a single dose administration of PYY, which is unusual in such studies (L. A. Campfield, Health SCOUT; Health Day News, September 3, 2004). The reported suppression of food intake also seems incongruent with the only slight reduction in hunger, suggesting that other factors such as nausea or alterations in food hedonics may have been involved. Nausea has been the most frequent side effect reported in human PYY studies to date (60). Based on the known strong potency of PYY to inhibit intestinal secretion (3,8), systemic infusion of PYY3-36 seems likely to cause obstipation. Obstipation, however, is known to stimulate satiety mechanisms mildly and transiently via vagal reflexes. A tendency toward decreased caloric content in faeces would be consistent with these actions (66). In a separate report, baseline PYY levels in an obese group were reported to be 40% lower than in a non-obese group (30). This is suggested to explain impaired satiety in this group, but another plausible explanation is that lower PYY in this group serves to trigger overeating (D. Cummings, Health SCOUT; Health Day News, September 3, 2004).

The crucial question is whether chronic peripheral infusion of PYY3-36 will decrease body fat mass in humans. PYY3-36 would have to override massive compensatory activity of numerous other orexigenic pathways (34). Although no scientific peer-reviewed information is available, Nastech Pharmaceutical Co., Inc., has developed a PYY3-36 nasal spray and has released the only report of reduced food intake and weight loss in PYY3-36-treated obese subjects (mean body mass index = 33.3; n = 37; six per treatment condition). An average decrease of 77 cal vs. 648 cal was reported for subjects given intranasal PYY3-36 before one meal a day vs. before all three meals a day for six consecutive days respectively, while an average weight loss of 1.3 pounds was reported only for those subjects on the maximum dosing condition over the 6-d study. However, it was not reported if that weight loss was the result of decreased fat mass, decreased muscle mass, or loss of water. While one other study reported decreased food intake in humans treated with PYY3-36 (29,30), this is the only press release reporting any body weight loss (67). Whether weight loss is sustained with repeated treatment remains to be determined. Chelikani et al. found rats to decrease food intake with continuous i.v. infusion of PYY3-36 (68), but no change in body weight was reported. Moran and colleagues recently found that, in primates, single doses of 4 and 12 µg (1 and 3 nmol)/kg PYY3-36 reduced food intake acutely but this suppression was not sustained despite multiple administrations over days (69). Unless the peptide substantially increases energy expenditure, a steady and sustained decrease of food intake will be critical to lasting weight loss. Still, although the large majority of studies in rodents did not evidence anorectic PYY3-36 effects, to date studies in humans and primates have yielded more promising results. Thus, failed rodent studies cast doubt on the value of PYY3-36 as a therapeutic agent, but in no way are they sufficient to rule out such putative value. Until there are more data in human and non-human primates, one cannot rule out possible species differences. Nevertheless, while such interspecies differences are conceivable, given the typical interspecies conservation of effects with respect to substances influencing ingestive behaviour, we find this somewhat unlikely.

**Conclusions**

This review aimed to critically evaluate all available information on a possible role of PYY3-36 as an endogenous
satiety agent and as an effective anti-obesity drug. Several observations have decreased initial enthusiasm about the potential of PYY3-36 as a magic bullet against the consequences of today’s hypercaloric environment. First, there is an unusually large number of failed attempts (we estimate about 90% of all studies) to replicate and extend the first reported anorectic effects of PYY3-36. Secondly, while the original studies presented a very elegant hypothesis and an intriguing collection of data (29), it is unfortunate that these original findings have not been reproduced by some 42 investigators and that very few supporting publications have appeared in the literature since then (over 3 years). In contrast, after the discovery of the ob/ob protein, leptin, though it was eventually found to vary in anorectic potency in some models of obesity (70), not a single group was reportedly unable to reproduce the original observations of reduced food intake and body weight. Moreover, despite the fact that leptin is incomparably more difficult to generate and more expensive to obtain, literally hundreds of studies have since supported the hormone’s catabolic role in energy balance. Thirdly, data are now emerging to indicate that basal PYY levels are apparently not abnormally low in obesity and that any small anorectic effects observed may have been evoked by visceral illness. Importantly, these data are being provided by independent laboratories, including laboratories of the authors of the original report of the anorectic actions of PYY3-36 (29).

Nevertheless, Batterham and colleagues have offered the scientific community an important set of observations. Their data suggesting a role for PYY3-36 as an endogenous anorectic substance brought attention to this peptide and provided an impetus for vigorous experimental efforts to reproduce and extend their results. The results of many subsequent investigations, however, have failed to confirm the original observations and have called the potency and, indeed, the effectiveness of PYY3-36 as an anorectic peptide into question. At this juncture, further clarity can best come from additional experimental and clinical data collected under the most rigorous conditions with sample sizes sufficient to yield definitive conclusions. However, in designing such studies, it may be worth considering possible explanations for why results appear so discrepant and, in that light, we offer the observations collected in this review article. We hope that the as-yet unexplained discrepancies in results serve to stimulate further research geared towards resolving the circumstances, if any, under which this peptide might produce consistent, physiologically meaningful, and behaviourally selective effects on human food intake and body fat mass.

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