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Peptide $YY_{3–36}$ Inhibits Food Intake in Mice through a Melanocortin-4 Receptor-Independent Mechanism

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Peptide $YY_{3–36}$ ($PYY_{3–36}$), a peptide released postprandially by the gut, has been demonstrated to inhibit food intake. Little is known about the mechanism by which $PYY_{3–36}$ inhibits food intake, although the peptide has been shown to increase hypothalamic proopiomelanocortin (POMC) mRNA *in vivo* and to activate POMC neurons in an electrophysiological slice preparation. Understanding the physiology of $PYY_{3–36}$ is further complicated by the fact that some laboratories have had difficulty demonstrating inhibition of feeding by the peptide in rodents. We demonstrate here that, like cholecystokinin, $PYY_{3–36}$ dose-dependently inhibits food intake by approximately 20–45% over a 3- to 4-h period post ip administration, with no effect on 12-h food intake. This short-lived satiety effect is not seen in animals that are not thoroughly acclimated to handling and ip injection, thus potentially explaining the difficulty in reproducing the effect. Surprisingly, $PYY_{3–36}$ was equally efficacious in inducing satiety in wild-type and melanocortin-4 receptor (MC4-R)-deficient mice and thus does not appear to be dependent on MC4-R signaling. The expression of c-Fos, an indirect marker of neuronal activation, was also examined in forebrain and brainstem neurons after ip treatment with a dose of $PYY_{3–36}$ shown to induce satiety. The peptide induced no significant neuronal activation in the brainstem by this assay, and only modest activation of hypothalamic POMC neurons. Thus, unlike cholecystokinin, $PYY_{3–36}$-induced satiety is atypical, because it does not produce detectable activation of brainstem satiety centers and is not dependent on MC4-R signaling. (*Endocrinology* 145: 2585–2590, 2004)

TWO ENDOGENOUS FORMS of peptide YY ($PYY_{1–36}$ and $PYY_{3–36}$) are synthesized by the gastrointestinal (GI) tract (1) and released into the circulation after a meal (2). They are released such that approximately 60% is $PYY_{1–36}$ and the rest is $PYY_{3–36}$ (3). Both peptides have a number of local effects on the GI system (4, 5) and have orexigenic actions when administered centrally (6, 7). Extensive studies of the effects of $PYY_{1–36}$ on food intake with respect to its site of action have shown a differential effect on its ability to increase food intake; intracerebroventricular injections into the fourth ventricle have a much greater effect of stimulating food intake compared with third ventricle $PYY_{1–36}$ administration (8). In agreement with these studies, reports have shown that $PYY_{1–36}$ predominantly exerts its orexigenic effects via the brainstem (8). However, elevated systemic levels of $PYY_{1–36}$, due to a gastric bypass surgery or peripheral injections, have emetic effects, leading to a reduction of food intake (9, 10).

The $PYY_{3–36}$ form appears to be anorexigenic when given peripherally (11, 12). In a recent study Batterham and coworkers (11) showed that ip injections of $PYY_{3–36}$, acting through Y2 receptors, can suppress fast-induced feeding in rats and mice. Additionally, $PYY_{3–36}$ was shown to activate proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARC) when bath-applied to hypothalamic slices *in vitro* (11). However, a number of laboratories (13) have had difficulty reliably and reproducibly repeating the anorexigenic effect of $PYY_{3–36}$ in the rodent.

One model for the anorexigenic action of $PYY_{3–36}$ proposes activation of ARC POMC neurons of the central melanocortin system (11), and indeed, $PYY_{3–36}$ administration has been demonstrated to elevate hypothalamic POMC mRNA levels (12); however, we were concerned by the very low percentage (20% with $PYY_{3–36}$ *vs.* 8% with saline) of POMC ARC neurons activated by $PYY_{3–36}$, as assessed by c-Fos immunohistochemistry. In this study we demonstrate a reproducible protocol for assessing the anorexigenic activity of $PYY_{3–36}$ utilizing a long acclimatization of animals, and that the peptide retains full activity in the melanocortin-4 receptor knockout ($MC4-R^{-/-}$) mouse.

Materials and Methods

Animals

$MC4-R^{-/-}$ and POMC-enhanced green fluorescent protein (EGFP) mice were derived from the animals described previously (14, 15) and were bred 10 generations into the C57BL/6J background. All transgenic animals were raised in group housing with their siblings and maintained at 23 ± 1 C on a 12-h light, 12-h dark cycle (0700–1900 h light). Mice were allowed *ad libitum* access to standard chow pellets (Purina Laboratory Rodent Diet 5001, Ralston Purina Co., St. Louis, MO; ~4.5% fat). Wild-type (WT) controls of the C57BL/6J strain were purchased to be age, sex, and weight matched (The Jackson Laboratory, Bar Harbor, ME). Upon arrival WT mice were allowed to acclimate for 1 wk under the conditions stated above. All studies were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the animal care and use committee of Oregon Health and Science University.

Source of reagents/peptides

All experiments, unless stated otherwise, were performed with human $PYY_{3–36}$ purchased from American Peptides (Sunnyvale, CA; first batch: lot Q08111T1). A second human $PYY_{3–36}$ batch and the synthetic

Abbreviations: ARC, Arcuate nucleus of the hypothalamus; CCK, cholecystokinin; EGFP, enhanced green fluorescent protein; GI, gastrointestinal; MC4-R, melanocortin-4 receptor; NTS, nucleus tractus solitarius; POMC, proopiomelanocortin; WT, wild-type.

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MC3-R/MC4-R antagonist SHU9119 were purchased form Bachem (Torrance, CA; lot 0558311), and a second independent batch of human $PYY_{3–36}$ was purchased from American Peptides (lot R05026T1). All peptides were certified by the manufacturer and came with HPLC data showing a single peak with the correct molecular weight from mass spectrograms and a purity greater than 97%. Peptides were dissolved in sterile isotonic saline and injected ip in a total volume of 500 μl/injection. Fresh human $PYY_{3–36}$ and SHU9119 concentrations were prepared on the day of injection from frozen stock solutions.

Feeding protocols

Response of unacclimated animals to a 16-h fast. Age-matched WT (The Jackson Laboratory) male mice (8 wk old) were used for unacclimated feeding studies. Mice were individually housed the day before a nocturnal fast (1800–1000 h). Injections and food consumption measurements were performed in a double-blinded experiment. Animals were injected ip at 1000 h with either saline or $PYY_{3–36}$ at a dose of 0.3, 3, or 10 μg/100 g. Food intake was measured hourly for the first 4 h and at 12 h, by placing two pellets of chow in petri dishes at the bottom of the cage at the time of ip injection. To minimize error attributable to loss of food particles, all bedding was screened before and after the experiment to capture any spilled food. Food in petri dishes was also screened to remove any bedding or other debris.

Response of acclimated animals to a 16-h fast. Age-matched WT (The Jackson Laboratory) male mice (8 wk) were used for the feeding study. Mice were individually housed for 1 wk. In the following week they were acclimated to daily ip saline injections at 1000 h with two pellets of food being placed in a petri dish on the floor of the cage and weighed hourly for 4 h. Animals were fasted for 16 h the night before the experiment (1800–1000 h). Food intake was measured by placing two pellets of chow in petri dishes on the floor of the cage at the time of ip injection (double-blinded) of either saline or $PYY_{3–36}$ at a dose of 0.3, 3, or 10 μg/100 g, and cumulative food intake was measured hourly for 4 h post injection. As before, to minimize error attributable to loss of food particles, all bedding and petri dishes were screened.

Response of acclimated animals in a nighttime feeding protocol. Age-matched $MC4\text{-}R^{-/-}$ and WT (The Jackson Laboratory) male mice (8 wk old) were used for the nocturnal feeding study. For habituation, mice were individually housed for 1 wk and injected with daily (500 μl) saline immediately before lights out (1900 h). Two pellets of food were placed in a petri dish on the floor of the cage immediately after injection, and food intake was measured hourly for 4 h. Animals were habituated until their food intake stabilized for at least 4 consecutive days before experimental treatment. Animals were randomly injected with either saline or 0.3 μg/100 g $PYY_{3–36}$ on the first experimental day, with 3 μg/100 g on the second day, and with 10 μg/100 g on the last experimental day. On nonexperimental days animals were injected with saline to measure deviation from previous habituation baseline. Measurement error was minimized with careful screening of petri dishes for debris and cage bedding for spilled food.

c-Fos immunohistochemistry

POMC-EGFP mice (23–27 g), a transgenic strain in which EGFP is expressed under the control of the POMC promoter (14), were handled and received 100 μl sterile saline, ip, at 0900 h for 5 d before the experiment to minimize background c-Fos immunoreactivity caused by stress. Animals received an ip injection of $PYY_{3–36}$ (5 μg/100 g) or sterile saline 90 min before being deeply anesthetized and undergoing transcardial perfusion with 0.9% heparinized saline, followed by 4% paraformaldehyde in 0.01 M PBS. Sections were cut at 30 μm from perfused brains and stored free-floating in 0.01 M PBS containing 0.03% sodium azide. The sections were incubated for 1 h at room temperature in blocking reagent (5% normal donkey serum in 0.01 M PBS and 0.3% Triton X-100). After the initial blocking step, the sections were incubated in rabbit anti-c-Fos antibody (sc-052, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) diluted 1:6000 in blocking reagent for 24 h at 4 C, followed by incubation in 1:500 donkey antirabbit Alexa 594 (Molecular Probes, Inc., Eugene, OR) for 1 h at room temperature. In the nucleus tractus solitarius (NTS) sections, the POMC-EGFP cells were detected using a 1:4000 dilution of rabbit anti-GFP antibody directly conjugated to Alexa 488 (Molecular Probes, Inc.). Between each stage the sections were washed thoroughly with 0.01 M PBS. At the end of the incubations the sections were mounted onto gelatin-coated slides, coverslipped using gel-based fluorescence mounting medium (Biomeda Corp., Foster City, CA), and viewed under a fluorescence microscope (Axioplan 2, Zeiss, Inc., Thornwood, NY). The number of c-Fos-immunoreactive cells was counted on sections that also contained POMC-EGFP cells by a person blinded to the individual treatments.

Statistics

Statistical analyses were performed using PRISM (GraphPad, San Diego, CA). Data are expressed as the mean ± SEM. One-way ANOVA with Dunnett's test *post hoc* test was used to determine significance in Fig. 1. Significance was determined using an unpaired (two-tailed) *t* test in Figs. 2 and 3. Significance was taken as $P < 0.05$.

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Results

$PYY_{3–36}$ does not reduce food intake after a single ip injection in 16-h fasted naive WT mice

We postulated that the inability of some laboratories to reduce food intake with a single ip injection of $PYY_{3–36}$ in mice (13) could be due to stress caused by the experimental procedure (16). To test this hypothesis we individually housed naive/unacclimated WT male mice at 1700 h and fasted them for 16 h (1800–1000 h) before a single ip injection of either saline or 0.3, 3, or 10 μg/100 g $PYY_{3–36}$ in a double-blinded experiment. $PYY_{3–36}$ did not reduce food intake at any dose or any time point (Fig. 1A). Unacclimated WT saline controls (Fig. 1A) ate 32.0% (Fig. 1B) and 53.5% (Fig. 1, C and D) less than acclimated WT saline controls in the first hour of measurement [unacclimated, 0.48 ± 0.13 g (Fig. 1A); acclimated, 0.71 ± 0.04 g (Fig. 1B); acclimated, 1.04 ± 0.08 g (Fig. 1, C and D)].

$PYY_{3–36}$ reduces food intake in a dose-dependent manner after a single ip injection after 16-h fast in acclimated mice

Due to our previous experience we tested $PYY_{3–36}$ action after a week-long acclimatization protocol that we have shown to reduce stress due to handling (16). In this study WT animals were individually housed for 1 wk and treated daily with ip saline injections, and food was weighed at the appropriate times for acclimation. The animals were fasted for 16 h before ip injection of $PYY_{3–36}$ at varying doses of 0.3, 3, and 10 μg/100 g, and cumulative food intake was measured at 1-h intervals for 4 h. Compound administration and food intake measurements were performed using a double-blind procedure to prevent any handling artifacts or experimental bias. $PYY_{3–36}$ reduced food intake in a dose-dependent matter in the first 4 h after administration (Fig. 1B). $PYY_{3–36}$ significantly reduced food intake in the 3 and 10 μg/100 g treatment groups compared with the saline-treated animals at both 1 and 2 h (Fig. 1B). Although there was a trend toward a reduction in food intake by $PYY_{3–36}$ with the 0.3 μg/100 g dose, statistical significance was not reached (Fig. 1B). Four hours after the ip injections, only the 10 μg/100 g dose retained its ability to significantly reduce food consumption (Fig. 1B). At 12 h post injection, no difference in food intake was measured at any concentrations (Fig. 1B, *inset*).

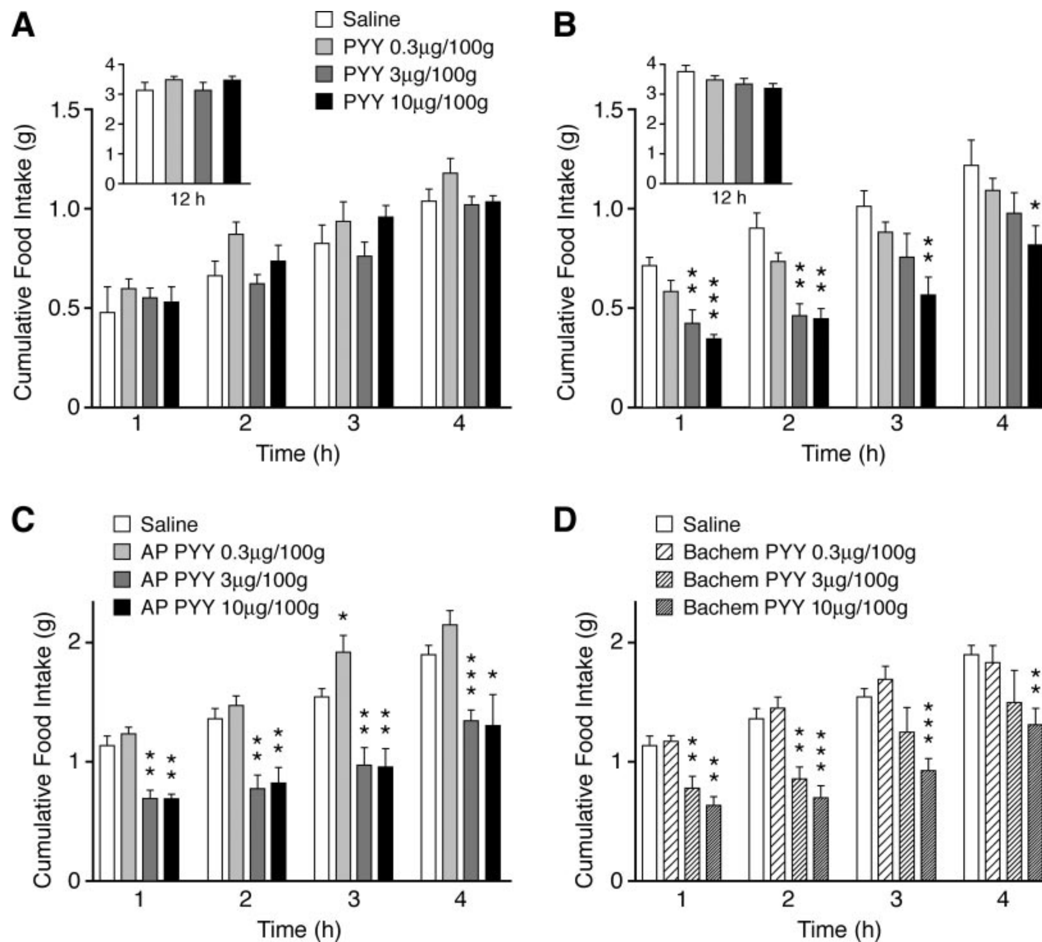

FIG. 1. $PYY_{3–36}$ reproducibly inhibits food intake in a dose-dependent manner after a 16-h fast in acclimated WT mice. A, Feeding response to increasing doses of $PYY_{3–36}$ at 1, 2, 3, 4, and 12 h after ip injection in unacclimated WT mice (n = 5). B, Dose-dependent inhibition of food intake at 1, 2, 3, and 4 h after ip $PYY_{3–36}$ injection in WT mice acclimated for 1 wk (n = 5). No effect of $PYY_{3–36}$ is seen at 12 h after ip injection (*inset*). C, Dose-dependent inhibition of food intake in acclimated WT mice with $PYY_{3–36}$ (American Peptides, batch 2) at 1, 2, 3, and 4 h after ip injection (saline, n = 10; 0.3 μg/100 g, n = 5; 10 μg/100 g, n = 4). D, Dose-dependent inhibition of food intake in acclimated WT mice with $PYY_{3–36}$ (Bachem) at 1, 2, 3, and 4 h after ip injection (saline, n = 10; 3 μg/100 g, n = 4; 10 μg/100 g, n = 5). Data are expressed as the mean ± SEM. By one-way ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

$PYY_{3–36}$ from different batches and companies reliably reduces food intake after a 16-h fast in acclimated WT mice in a dose-dependent manner

Given the small degree of anorexia caused by $PYY_{3–36}$ and the limited duration of its action, we postulated that negative results might be due to batch to batch differences in the peptide. We next tested the effects of two different batches of $PYY_{3–36}$ obtained from two separate manufacturers on reducing food intake by a double-blinded experiment in 16-h fasted WT mice acclimated to handling. Peptides from both companies reduced food intake in habituated animals in a dose-dependent fashion (Fig. 1, C and D) Bachem $PYY_{3–36}$ showed a dose-response curve with increasing doses of peptide (Fig. 1D), whereas American Peptides $PYY_{3–36}$ showed a saturation of its effects past the 3 μg/100 g dose (Fig. 1C). Additionally, $PYY_{3–36}$ from Bachem also acted in a short-term manner, where it reduced food intake significantly only for the first 2 h after injection (Fig. 1D) like American Peptides $PYY_{3–36}$ in Fig. 1B. As before (Fig. 1B), both new batches of peptides were unable to reduce food intake at the lowest dose (0.3 μg/100 g) at any time point measured, and, in fact, at the single time point of 3 h American Peptides $PYY_{3–36}$ stimulated feeding (Fig. 1C). At 10 μg/100 g, peptides from both companies significantly reduced food intake for the duration of the experiment (Fig. 1, C and D).

It is possible that the reduction in food intake that was observed in the previous experiments could have simply been due to ip injection of any peptide or from a nonspecific compound that the particular company uses for its peptide storage. To test this hypothesis we obtained the MC3/4R antagonist SHU9119 from Bachem simultaneously with $PYY_{3–36}$. The ip injections of SHU9119 (3 and 10 μg/100 g) in a double-blinded experiment performed in 16-h fasted WT acclimated mice had no effect on food intake at any time point that $PYY_{3–36}$ dose-dependently reduced food intake (data not shown).

MC4-R is not required for inhibition of feeding by $PYY_{3–36}$

$PYY_{3–36}$ increases POMC neuronal firing in hypothalamic slices (11) and POMC mRNA expression acutely after peripheral $PYY_{3–36}$ administration (12). However, ip injection of

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PYY_{3-36} only increases the expression of c-Fos, an indirect marker of neuronal activation, in approximately 12% of ARC POMC neurons (11). These results suggested that the role of the melanocortin system in the action of PYY_{3-36} needed to be examined more carefully. To test the role of the melanocortin system in the action of PYY_{3-36}, we evaluated the ability of MC4-$R^{-/-}$ mice to show an anorexigenic response after ip administration of PYY_{3-36}. We chose a nocturnal feeding response to PYY_{3-36} because it is most physiologically relevant. WT and MC4-$R^{-/-}$ feeding *ad libitum* were injected with either saline or PYY_{3-36} at increasing doses immediately before lights out (1900 h), and cumulative food intake was measured hourly for 4 h. The freely night-feeding WT animals showed a similar response to increasing doses of PYY_{3-36} as fasted animals (Fig. 1). At the lowest dose, PYY_{3-36} (0.3 μg/100 g) did not significantly reduce nocturnal food intake in WT mice over the 4 h of measurement (Fig. 2A). At the 3 μg/100 g dose, PYY_{3-36} reduced food intake significantly in WT over the first 3 h (Fig. 2B). At the highest dose of 10 μg/100 g, PYY_{3-36} significantly reduced food intake in WT for the 4 h of measurements (Fig. 2C).

MC4-$R^{-/-}$ mice injected with PYY_{3-36} exhibited a similar dose-dependent decrease in *ad libitum* nocturnal food intake as WT animals (Fig. 2). Like WT animals, the lowest dose of PYY_{3-36} (0.3 μg/100 g) did not induce a significant decrease in food intake in MC4-$R^{-/-}$ mice at any of the time points measured (Fig. 2A). The intermediate dose of PYY_{3-36} (3 μg/100 g) transiently reduced food intake in MC4-$R^{-/-}$ mice to a similar degree and over the same time course as in WT mice (Fig. 2B). At the highest dose of 10 μg/100 g PYY_{3-36}, MC4-$R^{-/-}$ animals responded in the same way as WT mice (Fig. 2C).

Daytime peripheral PYY_{3-36} administration induces c-Fos expression in POMC neurons in the ARC

To further examine the possible role of POMC neurons in mediating the effects of PYY_{3-36}, we performed a preliminary experiment to examine the activation of POMC neurons in both the ARC and NTS using c-Fos immunohistochemistry. Immunohistochemical experiments were performed using a previously characterized transgenic mouse in which EGFP is expressed under the control of the POMC promoter (14); thus, EGFP immunoreactivity was used to visualize POMC-positive cells. Daytime ip injection at 0900 h of PYY_{3-36} at 5 μg/100 g significantly increased c-Fos expression in POMC neurons in the ARC from 9% to 22% (Fig. 3A) similar to data reported previously (11). No increase was seen in c-Fos-positive POMC neurons in the NTS; however, this analysis involved a small sample (three animals) examined under a single condition (Fig. 3C). In this preliminary experiment there was a trend upward, but no significant increase in total c-Fos expression in the ARC (Fig. 3B) or NTS (Fig. 3D) was found compared with saline-treated animals.

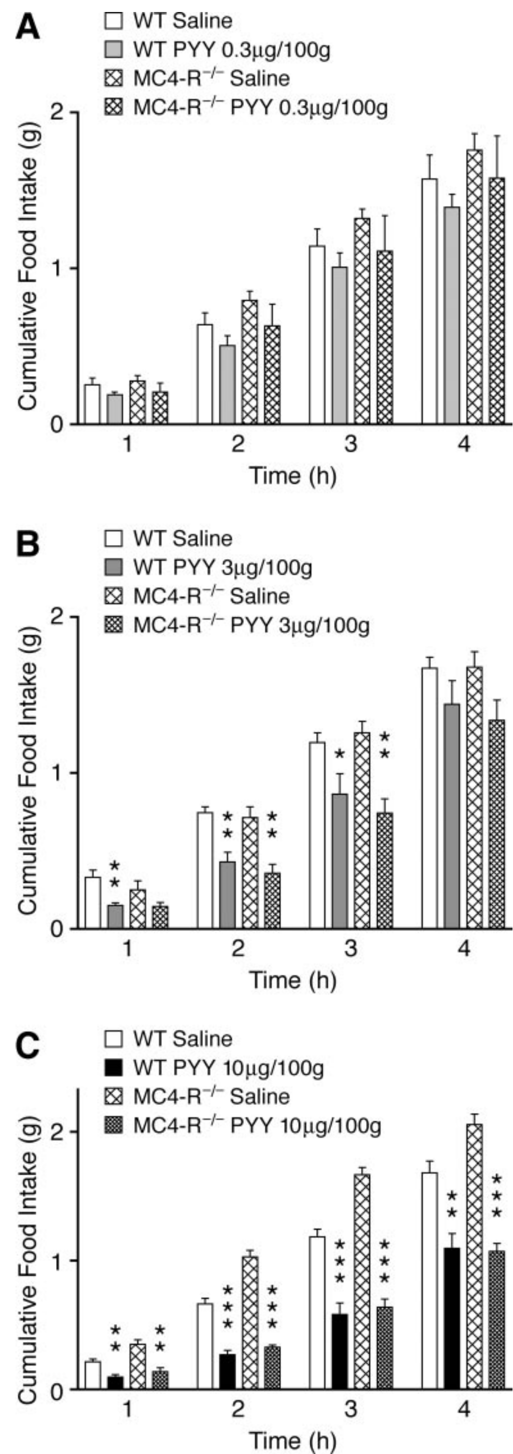

FIG. 2. MC4-$R^{-/-}$ mice and WT mice respond equivalently to increasing concentrations of PYY_{3-36} in a nocturnal feeding paradigm. A, Nocturnal feeding responses of WT and MC4-$R^{-/-}$ mice to a PYY_{3-36} dose of 0.3 μg/100 g at 1, 2, 3, and 4 h post injection (WT saline and 0.3 μg/100 g, n = 6; MC4-$R^{-/-}$ saline, n = 6; 0.3 μg/100 g, n = 5;). B, Nocturnal feeding responses of WT and MC4-$R^{-/-}$ mice to a PYY_{3-36} dose of 3 μg/100 g at 1, 2, 3, and 4 h post injection (WT saline, n = 6; 3 μg/100 g, n = 5; MC4-$R^{-/-}$ saline and 3 μg/100 g, n = 6). C, Nocturnal feeding responses of WT and MC4-$R^{-/-}$ mice to a PYY_{3-36} dose of 10 μg/100 g at 1, 2, 3, and 4 h post injection (all animals, n = 6). Data are expressed as the mean ± SEM. By two-tailed *t* test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

In this study we examined the effects of PYY_{3-36} on food intake in mice during daytime feeding after a 16-h fast and during normal nighttime feeding. We show here that in both paradigms PYY_{3-36} dose-dependently reduces food intake in

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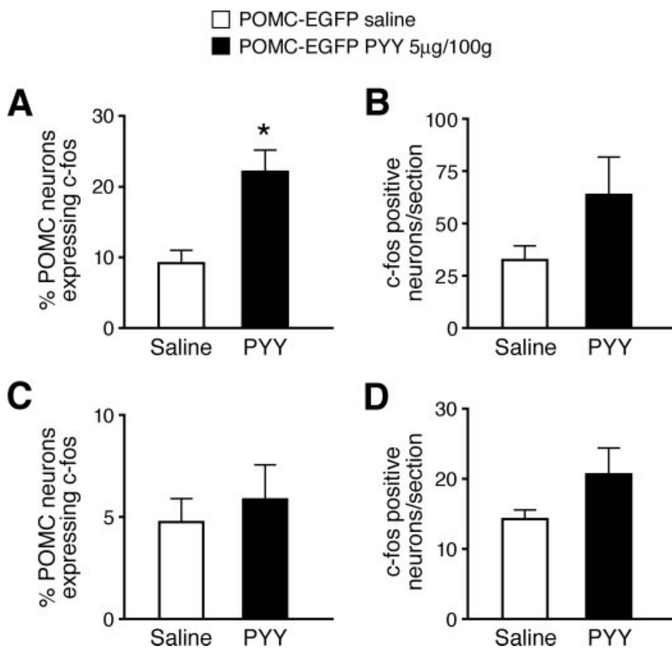

FIG. 3. PYY_{3-36} at dose of 5 μg/100 g activates POMC neurons after 90 min in the ARC, but not in the NTS of the brainstem. A, Approximately 22% of POMC neurons of the ARC are activated by ip administration of PYY_{3-36} compared with 9% by saline. B, There was no significant increase in c-Fos expression in total ARC after ip PYY_{3-36} treatment. C, POMC neurons of the NTS were not activated by PYY_{3-36} treatment. D, There was no significant increase in c-Fos expression in NTS neurons after ip administration of PYY_{3-36}. Saline, n = 4; 5 μg/100 g, n = 3. Data are expressed as the mean ± SEM. By two-tailed t test: *, $P < 0.05$.

acclimated animals. Furthermore, the degree and duration of food reduction are similar at the same doses of PYY_{3-36} administered from different batches and manufacturers. At the lowest dose of peptide (0.3 μg/100 g), a trend in food reduction was seen in the first and second hours of measurement, which did not reach statistical significance. At the higher dose of the peptide (3 μg/100 g), a significant reduction in food intake was observed during the first 2 h in the daytime experiments and during the first 3 h of the nocturnal experiment. However, the inhibition of food intake by PYY_{3-36} was short-lived. At the dose of 3 μg/100 g, the effect of PYY_{3-36} was reversed by a rebound hyperphagia by the fourth hour post injection, as indicated by the loss of significance in cumulative food intake between the second and third hours in daytime experiments and between the third and fourth hours in the nocturnal experiments. This trend was further observed at the highest dose of PYY_{3-36} (10 μg/100 g), but the reduction in food intake at this dose remained significant for the duration of the experiment. By 12 h post injection, no difference in food intake was seen between treatments at any concentration studied in the daytime 16-h fasted experiment. The results of these experiments would argue that PYY_{3-36} has a similar mechanism of action in its ability to reduce food intake after a fast or during physiologically relevant nocturnal feeding. The short duration of action of PYY_{3-36} and its inability to reduce 12-h food intake are reminiscent of the action of the satiety factors cholecystokinin (CCK) and bombesin (17, 18).

Recently, there have been reports that PYY_{3-36} is incapable of reliably reducing food intake (13). Here we showed that without proper habituation of the WT mice, the satiating effect of PYY_{3-36} was not evident. This loss of efficacy may be due to the effects of stress caused by handling and ip injection, as evident by the 32% decrease in food intake in unacclimated WT mice compared with acclimated WT mice (16). Therefore, as PYY_{3-36} has a modest anorexigenic efficacy and a short time window of action, its effects may be insignificant relative to the prominent reduction in food intake due to stress. Alternatively, if PYY_{3-36} and stress activate common anorexigenic circuits, stress-induced anorexia could mask the effects of PYY_{3-36}. The PYY_{3-36}-induced reduction in food intake is small even at high doses, and the effect is very short-lived. Thus, the action of PYY_{3-36} could also be easily missed if food intake is not measured within the first 4 h after injection or if the peptide is administered sc *vs.* ip. For example, a study by Challis *et al.* (12) showed that PYY_{3-36} (10 μg/100 g) reduced food intake after a 24-h fast, but did not measurably reduce nocturnal food intake in nonfasted, freely feeding mice when food intake was measured 6 h post injection. The lack of response seen with this paradigm may be due to the fact that food intake was not measured until 6 h after peptide administration. In our study the effect of PYY_{3-36} on food intake was largely absent by this time point. Furthermore, it is not clear whether these animals were acclimated to handling before the procedure, but this may be a contributing factor to the lack of response seen.

Additionally, different batches of peptide from American Peptides and Bachem both similarly and reliably reduced fast-induced food intake in the first 4 h of measurement in a double-blinded experiment, which was used to prevent any handling artifacts.

In this study we further tested the hypothesis that PYY_{3-36} acts via the central melanocortin system. The original report by Batterham and colleagues (11) showing that PYY_{3-36} has anorexigenic effects in mice also proposed that these effects might be mediated by the central melanocortin system. They observed an increase in POMC neuron firing with PYY_{3-36} in an electrophysiological slice preparation and an increase in c-Fos expression in POMC neurons of the ARC. We wanted to further examine this model in two ways: 1) by examining whether the MC4-R is essential for the anorexigenic action of PYY_{3-36}, and 2) by addressing whether POMC neurons in the ARC are activated by PYY_{3-36} and whether a potent activation of brain stem neurons by PYY_{3-36} is seen, as is the case with CCK or gastric distention (20–23). Here we show that MC4-$R^{-/-}$ mice are just as responsive to the anorexigenic effects PYY_{3-36} as WT mice in a nocturnal feeding paradigm. This result argues that the MC4-R is not essential for the anorexigenic action of PYY_{3-36}. Therefore, although the increase in the POMC neuron firing rate in slice preparations (11) and the induction of POMC mRNA (12) by PYY_{3-36} may indeed lead to an increase in melanocortin signaling though the MC4-R, this does not appear to be essential for the anorexigenic effects of the peptide.

In our preliminary immunohistochemical study, a small, but significant, increase in of c-Fos was seen in ARC POMC neurons after daytime administration of PYY_{3-36}, consistent with previously published results (11). Surprisingly, only a

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small nonsignificant increase in c-Fos immunoreactivity-positive cells was observed in total NTS neurons and NTS POMC neurons. The relatively small increase in c-Fos expression in the ARC and the lack of a significant increase in NTS may be due to a number of factors. First, the actions of PYY_{3-36} via the autoinhibitory Y2 receptor may indirectly modulate anorexigenic neurons and thus may not be potent enough to activate c-Fos; for example, in the ARC PYY_{3-36} appears to stimulate POMC neurons by indirectly decreasing the inhibitory γ-aminobutyric acid-ergic drive onto POMC neurons. Alternatively, PYY_{3-36} may inhibit feeding via a mechanism quite distinct from other gut-derived satiety factors. The lack of significant c-Fos expression in the NTS after PYY_{3-36} administration is in contrast to other GI peptides such as CCK and bombesin (24, 25), which also have short-term satiety-like actions yet induce c-Fos immunoreactivity in a great number of NTS neurons (24, 25). We have recently demonstrated that ip CCK routinely activates greater than 30% of POMC NTS neurons under similar experimental conditions, and furthermore, that the anorexic actions of CCK are blocked by either genetic or pharmacologic MC4-R blockade (26). Regardless of the causes behind the limited up-regulation of c-Fos in NTS and ARC after inhibition of feeding by PYY_{3-36}, the data presented here argue that PYY_{3-36} inhibits food intake via a strikingly different mechanism than that of either the long-term adipostatic factor leptin, which can potently activate ARC POMC neurons (27), or satiety signals, such as CCK, bombesin, or gastric distension, which potently activate c-Fos in NTS neurons.

In summary, we have presented data indicating that the satiating effect of PYY_{3-36} is complex, atypical, and does not require the presence of the MC4-R. If the MC4-R is not essential, other mechanisms must be involved. They may include atypical mechanisms of action of PYY_{3-36} on POMC neurons, other sites of action of PYY_{3-36} in the central nervous system, or, finally, a possible peripheral mode of action.

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