Peptide YY $(PYY)_{3–36}$ modulates thyrotropin secretion in rats

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Abstract

Peptide YY $(PYY)_{3-36}$ is a gut-derived hormone, with a proposed role in central mediation of postprandial satiety signals, as well as in long-term energy balance. In addition, recently, the ability of the hormone to regulate gonadotropin secretion, acting at pituitary and at hypothalamus has been reported. Here, we examined PYY_{3-36} effects on thyrotropin (TSH) secretion, both *in vitro* and *in vivo*. PYY_{3-36}-incubated rat pituitary glands showed a dose-dependent decrease in TSH release, with 44 and 62% reduction at 10^{-8} and 10^{-6} M ($P<0{\cdot}05$ and $P<0{\cdot}001$ respectively), and no alteration in TSH response to thyrotropin-releasing hormone. *In vivo*, PYY_{3-36} i.p. single injection in the doses of 3 or 30 μg/kg body weight, administered to rats fed *ad libitum*, was not able to change serum TSH after 15 or 30 min. However, in fasted rats, PYY_{3-36} at both doses elicited a significant rise (approximately twofold increase, $P<0{\cdot}05$) in serum TSH observed 15 min after the hormone injection. PYY_{3-36} treatment did not modify significantly serum T_4, T_3, or leptin. Therefore, in the present paper, we have demonstrated that the gut hormone PYY_{3-36} acts directly on the pituitary gland to inhibit TSH release, and in the fasting situation, *in vivo*, when serum PYY_{3-36} is reduced, the activity of thyroid axis is reduced as well. In such a situation, systemically injected PYY_{3-36} was able to acutely activate the thyrotrope axis, suggesting a new role for PYY_{3-36} as a regulator of the hypothalamic–pituitary–thyroid axis.

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Introduction

Peptide YY $(PYY)_{3-36}$ is a gut-derived hormone, produced by endocrine L cells lining the distal small bowel and colon, released postprandially in proportion to the calories ingested (Adrian *et al.* 1985). Peripheral administration of PYY_{3-36} in rodents or humans has been reported to induce a marked inhibition of food intake (Batterham *et al.* 2002, 2003). PYY_{3-36} inhibits food intake at plasma concentrations that are within the normal physiological range seen in man after meals (Batterham *et al.* 2002, 2003) and therefore, a role for the hormone in central mediation of postprandial satiety signals has been proposed. Moreover, long-term administration has been demonstrated to induce a reduction in body weight (Batterham *et al.* 2002, 2003) although this is still a matter of controversy (Tschöp *et al.* 2004).

In addition, recent studies suggested that PYY_{3-36} may act as a neuroendocrine regulator. It has been demonstrated in prepubertal rats that PYY_{3-36} stimulated prolactin, luteinizing hormone (LH), and FSH secretion acting directly at pituitary gland, although it seems to have an inhibitory action at the hypothalamic level (Aguilar *et al.* 2004, Fernandez-Fernandez *et al.* 2005). Therefore, similar to other hormones involved in energy homeostasis, PYY_{3-36} is also able to influence the reproductive axis. However, there is no information concerning the thyrotrope axis.

PYY_{3-36} is a member of the neuropeptide Y (NPY) family and it is an agonist of receptor subtypes NPY-Y2 and NPY-Y5 (Keire *et al.* 2000). Experimental evidence suggests that circulating PYY_{3-36} inhibits appetite by acting directly on the arcuate nucleus via the Y2 receptor, a presynaptic inhibitory autoreceptor (Batterham *et al.* 2002, Talsania *et al.* 2005). NPY is a potent stimulator of food intake and PYY_{3-36} was able to inhibit the electrical activity of arcuate NPY neurons as well as to reduce the expression of the NPY mRNA (Batterham *et al.* 2002, Challisa *et al.* 2003). In addition, NPY originating in the hypothalamic arcuate nucleus exerts a profound inhibitory effect on the thyroid axis via effects on hypophysiotropic thyrotropin-releasing hormone (TRH) neurons. Chronic intracerebroventricular administration of NPY to normally fed rats resulted in reduction of circulating levels of thyroid hormones with inappropriately normal or low thyrotropin (TSH), and suppression of proTRH mRNA in the hypothalamic paraventricular nucleus (PVN; Fekete *et al.* 2001). NPY effects were reproduced by NPY-Y1 and NPY-Y5 analogs injected into the third cerebral ventricle, suggesting that both the receptors mediate NPY suppression of the hypothalamic–pituitary–thyroid axis (Fekete *et al.* 2002).

During fasting, as a means of conserving energy, the hypothalamic–pituitary–thyroid axis is suppressed and the activation of arcuate NPY neurons is a major component of the regulatory mechanism that causes a decline in proTRH mRNA in PVN, reducing circulating levels of TSH and thyroid hormones. Fasting is associated with reduced serum concentrations of PYY_{3-36} (Tovar *et al.* 2004, Chan *et al.* 2005), and peripherally injected PYY_{3-36} partly reversed the

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fasting-induced c-Fos expression in arcuate nucleus neurons of mice (Riediger *et al.* 2004), suggesting a role for the peptide in fasting adaptation.

Therefore, considering NPY effects on thyroid axis, and the presence of Y5 receptors, and the mRNA encoding Y2 and Y5 receptors in pituitary gland (Parker *et al.* 2000, Fernandez-Fernandez *et al.* 2005), we tested the hypothesis that PYY_{3-36} may act directly at the pituitary to modulate TSH secretion. In addition, we also investigated whether PYY_{3-36}, injected systemically, may modify the thyrotrope axis in fed and fasting rats.

Materials and Methods

Animals

Adult male Wistar rats, weighing 250–300 g, were kept under controlled lighting (12 h light:12 h darkness cycle, lights on at 0700 h) and controlled temperature (23±1 °C). All experimental protocols were approved by our institutional animal care committee.

In vitro *experiments*

Ad libitum fed rats were killed by decapitation, and their anterior pituitaries were quickly dissected out for *in vitro* testing as described before (Rettori *et al.* 1989, Ortiga-Carvalho *et al.* 2002) Each hemi-pituitary was immediately transferred to a flask containing 1 ml Krebs–Ringer bicarbonate medium (pH 7·4) at 37 °C in an atmosphere of 95% O_2/5% CO_2 in a Dubnoff metabolic shaker. After a 30-min preincubation period, medium was changed to 1 ml medium alone (control) or medium containing PYY_{3-36} (Bachem California, Inc., Torrance, CA, USA) to a final concentration of 10^{-10}, 10^{-8}, or 10^{-6} M. At the end of a 2 h incubation period, an aliquot was removed for TSH measurement. In another set of experiments, after 2 h incubation, a small aliquot was removed for TSH measurement and TRH (Bachem California, Inc.) was added to a final concentration of 50 nM in all tubes. The incubation was continued for 30 min to determine the TRH-stimulated TSH release in the absence or presence of the different concentrations of PYY_{3-36}.

In vivo *experiments*

Ad libitum fed rats were divided into three groups that received a single i.p. injection of 3 or 30 μg/kg body weight (BW) PYY_{3-36} or 0·2 ml saline vehicle (control group). Another set of rats was fasted for 3 days before they received a single i.p. injection of 3 or 30 μg/kg BW PYY_{3-36} or 0·2 ml saline vehicle (control group). In both experiments, rats were killed by decapitation, 15 or 30 min after the injection. Experiments were performed in the morning between 1000 and 1100 h. Serum was obtained from trunk blood for hormone measurements.

Quantification of TSH

TSH concentration in the serum and in the incubation medium was measured by specific RIA, employing reagents supplied by the National Institute of Diabetes, Digestive and Kidney Diseases, National Hormone & Peptide Program (NIDDK–NHPP) (Torrance, CA, USA), as previously described (Chard 1987, Ortiga-Carvalho *et al.* 1996), and was expressed in terms of the reference preparation 3 (RP3). Within-assay variation was 5·7%. Samples of the same experiment were measured within the same assay. Minimum assay detection was 0·52 ng/ml.

Quantification of serum concentrations of T_4, T_3, and leptin

Serum T_4 and T_3 were detected by RIA (MP Biomedicals Inc, Irvine, CA, USA). Detection limits were: 1 μg/dl for T_4 and 25 ng/dl for T_3. Serum leptin was measured using a specific rat RIA by LINCO Research (St Charles, MO, USA). Minimum detectable level was 0·5 ng/ml. Intraassay variation was less than 7% for all hormone measurements and all the samples were run within the same assay.

Statistical analysis

Data are reported as means±S.E.M. One-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test was employed for the assessment of significance of data. Serum TSH was analyzed after logarithmic transformation (Zar 1996). Differences were considered to be significant at $P<0·05$.

Results

In vitro *experiments*

PYY_{3-36}-incubated hemi-pituitary glands showed, after 2 h incubation, a dose-dependent decrease in TSH release, statistically significant at 10^{-8} M ($P<0·05$) and 10^{-6} M ($P<0·001$), with a reduction of 44 and 62% respectively (Fig. 1). However, the TSH response to TRH was not significantly different among groups, presenting an increment around four times above the basal levels (TSH before TRH) in all groups.

In vivo *experiments*

The i.p. administration of 3 or 30 μg/kg BW of PYY_{3-36} to *ad libitum* fed rats induced no changes in serum TSH either after 15 or 30 min (Fig. 2). Also, serum leptin and serum T_4 (Table 1) and T_3 concentrations (data not shown) were not significantly modified by the treatment with PYY_{3-36}. However, in fasted rats (Fig. 3), a significant rise in serum TSH was detected 15 min after PYY_{3-36} administration at both doses (approximately twofold increase, $P<0·05$). This

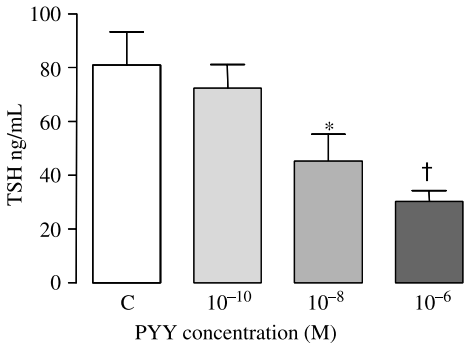

Figure 1 TSH release from isolated rat hemi-anterior pituitary glands incubated in absence (C, control) or presence of PYY_{3-36} at different concentrations (10^{-10}, 10^{-8}, 10^{-6} M) for 2 h. $n=10$ hemi-pituitaries per group. *$P<0{\cdot}05$, †$P<0{\cdot}001$ vs control group. Data represent means±S.E.M. Figure representative of two independent experiments.

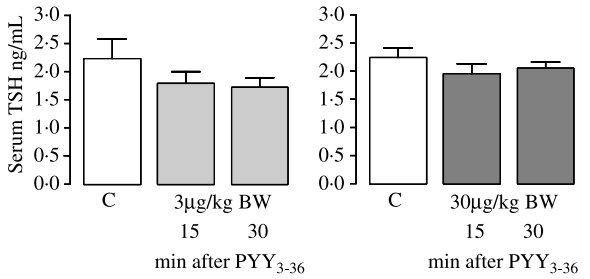

Figure 2 Serum TSH concentration in fed rats after intraperitoneal administration of PYY_{3-36} 3 (A) and 30 (B) µg/kg BW or saline (C). Determinations were performed at 15 and 30 min after injection of the peptide. $n=9$–10 animals per group. Data are presented as means±S.E.M.

effect was transitory, since no statistically significant effect was observed 30 min after PYY_{3-36} injection of both doses in fasted rats. Serum concentrations of T_4 and leptin were not changed by PYY_{3-36} treatment at any time point or doses (Table 1). Fasted rats presented approximately 11% reduction in body weight and in most of them serum T_3 was below 25 ng/ml.

Discussion

Here, we first demonstrated that PYY_{3-36} acts directly at the pituitary to inhibit TSH secretion. Y2 and Y5 mRNA receptors had been detected at low levels in pituitary gland (Parker *et al.* 2000, Fernandez-Fernandez *et al.* 2005), however, binding studies indicated the presence of Y5 (Parker *et al.* 2000) and the absence of Y2 receptors (Sheikh *et al.* 1998). Therefore, it is probable that PYY_{3-36} elicited the inhibitory effect on TSH secretion acting through activation of pituitary Y5 receptors. This would be consistent with the fact that an Y5 analog, injected centrally in rats, was able to suppress the hypothalamus–pituitary–thyroid axis. In addition, the present data are in agreement with a previous study showing that NPY, also a Y5 ligand, acts directly at rat pituitary to reduce β-TSH mRNA levels, as demonstrated by the incubation of pituitary cell cultures with NPY (Chowdhury *et al.* 2004). However, TRH action at the thyrotropes seems to be preserved, since the fold increment on TSH release induced by TRH was not significantly modified by the presence of PYY_{3-36} in the incubation medium.

Contrary to what could be expected in view of the inhibitory pituitary action of PYY_{3-36} on TSH release, systemic i.p. administration of the hormone in normally fed rats (3 and 30 µg/kg) failed to significantly modify serum TSH levels. Previous work of Fernandez-Fernandez *et al.* (2005), using a similar PYY_{3-36} administration protocol, was also unable to demonstrate *in vivo* effects of PYY_{3-36} on LH secretion, although the peptide had stimulated LH release from incubated rat pituitaries. Similar findings were reported concerning PYY_{3-36} effects on PRL secretion (Aguilar *et al.* 2004). The doses of PYY_{3-36} that we employed can be considered low and moderate, and it is possible that a higher dose would be necessary to modify TSH secretion. However, as demonstrated in previous studies (Batterham *et al.* 2002, Challisa *et al.* 2003, Talsania *et al.* 2005), similar PYY_{3-36} doses were sufficient to inhibit food intake in rats, suggesting that these doses were enough to activate arcuate nucleus Y2 receptors. An alternative explanation could be that the final effect of injected PYY_{3-36} may be the result of its action at different targets. In this sense, it must be considered that systemic PYY_{3-36} rapidly reaches the arcuate nucleus, an area partially outside the blood–brain barrier, where it binds with high affinity to autoinhibitory Y2 receptors in NPY neurons,

Table 1 Serum concentration of T4 and leptin in fed and fasting rats after intraperitoneal administration of peptide YY $(PYY)_{3-36}$. Data are presented as means±S.E.M. of two separated experiments (fed and fasting rats). $n=7$–10 per group.

		Control	PYY 3 µg 15 min	PYY 3 µg 30 min	Control	PYY 30 µg 15 min	PYY 30 µg 30 min
Group							
T_4 µg/dl	Fed	3·71±0·28	3·88±0·15	4·02±0·19	3·71±0·31	4·02±0·22	4·36±0·40
T_4 µg/dl	Fasted	2·86±0·28	2·62±0·10	2·18±0·05	2·76±0·18	2·67±0·30	2·37±0·19
Leptin ng/ml	Fed	2·64±0·25	2·40±0·16	3·03±0·41	3·91±0·30	3·56±0·44	3·80±0·32
Leptin ng/ml	Fasted	1·27±0·20	1·17±0·20	0·84±0·17	0·97±0·14	0·76±0·07	0·78±0·09

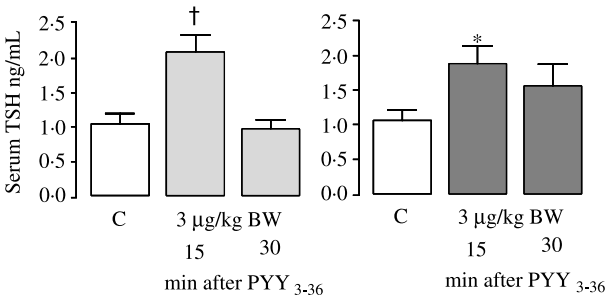

Figure 3 Serum TSH concentrations in fasted rats after intraperitoneal administration of PYY$_{3-36}$ 3 (A) and 30 (B) µg/kg BW or saline (C). Determinations were performed at 15 and 30 min after injection of the peptide. $n=8$–10 animals per group. $^{*}P<0{\cdot}05$, $^{\dagger}P<0{\cdot}001$ vs C (control vehicle-injected). Data are presented as means ± S.E.M.

which is the main postulated mechanism for its anorexigenic effect (Batterham *et al.* 2002). Therefore, by reducing NPY neuron activity, PYY$_{3-36}$ potentially may decrease NPY inhibitory input into TRH neurons. Thereby, it is possible that PYY$_{3-36}$ systemic administration had induced a higher TRH secretion that was counteracted by the direct pituitary inhibitory action, resulting in no alteration in serum TSH.

However, in fasted rats, it is likely that inhibition of NPY neurons via arcuate Y2 receptors is the predominant mechanism leading to the increase in serum TSH. In fasting conditions, there is a higher NPY inhibitory tonus on TRH neurons (Fekete *et al.* 2001) and, in addition, fasting was associated with reduced PYY$_{3-36}$ serum levels, as reported by others (Tovar *et al.* 2004, Chan *et al.* 2005), which may render the tissues more sensitive to PYY$_{3-36}$ action. Other reports support the concept that fasting enhances PYY$_{3-36}$ effects, such as those concerning the peptide action on gonadotropin secretion (Pinilla *et al.* 2006) and on food intake (Challisa *et al.* 2003, Riediger *et al.* 2004).

PYY$_{3-36}$ effect on TSH release was observed 15 min after the hormone administration, coincidently with previous reported time for peak PYY$_{3-36}$ serum levels after a single i.p. injection in the dose of 3 µg/kg BW (Batterham *et al.* 2002). This fast action is consistent with a central action of PYY$_{3-36}$ through activation of Y2 receptors in arcuate nucleus, since these receptors have high affinity for PYY$_{3-36}$. However, the effect was transitory, which may reflect the short-life of the peptide, and the fact that a single dose would not be enough to normalize the reduced levels of PYY$_{3-36}$ in fasting rats. However, it cannot be excluded that the activation of inhibitory Y5 receptors pathways in the pituitary may counteract the arcuate nucleus Y2-mediated stimulus in thyrotrope axis.

As expected, fasted rats exhibited lower serum thyroid hormone levels and leptin; however, the effect of PYY$_{3-36}$ on TSH release could not be attributed to changes in these hormone levels, since they remained similar among groups.

Reduction in serum leptin during fasting is believed to have a major role as a peripheral signal of energy insufficiency, resulting in an integrated response at the central nervous system which includes the activation of NPY-producing neurons in the hypothalamic arcuate nucleus. Considering that peripherally injected PYY$_{3-36}$ partly reversed the fasting-induced c-Fos expression in arcuate nucleus neurons of mice (Riediger *et al.* 2004), it is possible that reduction in serum PYY$_{3-36}$ also plays a role in neuroendocrine adaptation to fasting, which is an important component in the suppression of thyrotrope axis, a hypothesis that remains to be fully explored.

In conclusion, in the present paper we have demonstrated that the gut hormone PYY$_{3-36}$ acts directly at the pituitary gland to inhibit TSH release and that *in vivo*, systemically injected PYY$_{3-36}$ is able to acutely activate the thyrotrope axis during fasting. Therefore, we propose a new role for PYY$_{3-36}$ as a regulator of the hypothalamic–pituitary–thyroid axis.

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