Stimulation of NPY Y2 receptors by PYY3-36 reveals divergent cardiovascular effects of endogenous NPY in rats on different dietary regimens

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Running head: Cardiovascular effects of PYY3-36
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Abstract

In the present experiments the gut hormone peptide YY₃₋₃₆ (PYY₃₋₃₆) which inhibits neuropeptide Y (NPY) release, was used as a tool to study the cardiovascular effects of endogenous NPY under different dietary regimens in rats instrumented with a telemetry transmitter. In a first experiment rats were placed on a standard chow diet ad libitum and in a second experiment on a high fat diet ad libitum. After six weeks, PYY₃₋₃₆ (300 µg/kg) or vehicle were injected intraperitoneally. In a third experiment PYY₃₋₃₆ or vehicle were administered after 14 days of 50% restriction of a standard chow diet.

In food restricted rats, PYY₃₋₃₆ increased mean arterial pressure (+7 ± 1 mmHg, mean ± SEM, p<0.001 vs. saline, One Way Repeated Measures ANOVA with Bonferroni t-test) and heart rate (+22 ± 4 beats/min, p<0.001) during 3 hours after administration. Conversely, PYY₃₋₃₆ did not influence mean arterial pressure (0 ± 1 mmHg) and heart rate (-8 ± 5 beats/min) significantly in rats on a high fat diet. Rats fed standard chow diet ad libitum showed an intermediate response (mean arterial pressure +4 ± 1 mmHg, p<0.05 and heart rate +5 ± 2 beats/min, not significant).

Thus, in our studies divergent cardiovascular responses to PYY₃₋₃₆ were observed in rats on different dietary regimens. These findings suggest that the cardiovascular effects of PYY₃₋₃₆ depend on the hypothalamic NPY release, which is increased after chronic food restriction and decreased during a high fat diet.
Introduction

Neuropeptide Y (NPY) is one of the strongest endogenous stimulators of food intake (1) and is abundantly expressed in hypothalamic feeding centers such as the arcuate (ARC) and paraventricular nucleus (PVN) (2, 3). It has been demonstrated that the hypothalamic content of NPY varies with nutritional state (4) probably due to different NPY release (5, 6). Besides its role in appetite control, NPY also decreases blood pressure, heart rate and plasma norepinephrine levels after injection into the PVN (7, 8, 9) or the nucleus of the solitary tract (NTS) (10). Consequently, NPY-containing neurons that project from hypothalamic feeding centers (e.g. ARC, PVN) to cardiovascular centers of the brainstem (e.g. NTS) might be involved in both the regulation of energy balance and the regulation of cardiovascular function.

NPY and peptide YY (PYY) are closely related polypeptides, which are composed of 36 amino acids and share considerable homology (in rats: 67%) (11). While NPY acts as a neurotransmitter, the two endogenous forms of PYY (PYY_{1-36} and PYY_{3-36}) are gut derived hormones secreted by intestinal endocrine cells (L-cells) into the circulation after a meal (12, 13). PYY_{1-36} binds and activates at least three NPY receptor subtypes (Y1, Y2 and Y5) in rats and humans, whereas PYY_{3-36} is selective for the Y2 receptor (14). In a recent study it has been demonstrated that the gut hormone PYY_{3-36} physiologically inhibits food intake (15). In the present experiments, we used PYY_{3-36}, which inhibits NPY release by activation of presynaptic NPY Y2 receptors, as a tool to analyze the cardiovascular effects of endogenous NPY under different dietary regimens. NPY Y2 receptors are highly expressed on NPY neurons in the ARC, which is a brain area directly accessible to circulating hormones. For that reason we assumed that the peripheral administration of PYY_{3-36} might influence cardiovascular regulation by an ARC-PVN-NTS neuronal projection and that the magnitude of the cardiovascular PYY_{3-36} effects depend on the prevailing central NPY tone. To test this hypothesis conscious, unrestrained normotensive rats were studied under two extremes of caloric intake, namely chronic food restriction (high central NPY tone) and overfeeding with a high fat diet (low central NPY tone).

Materials and Methods

Animals. Ten week old male Sprague-Dawley rats were obtained from Iffa Credo, L’Arbresle, France. The rats were housed in individual plastic cages in a room with controlled temperature (21-22 °C) and a 12:12-h light-dark cycle (lights on from 06:00 a.m. to 06:00 p.m.). The rats were allowed one week to get accustomed to the new environment before the telemetry surgeries were performed. After a recovery period of two weeks post-surgery the different diet regimens were started. In a first experiment rats (n=7) were placed on a standard chow
diet (SCD) *ad libitum* (Nafag Ecosan, Switzerland, NAFAG 3432, 3.0 kcal/g, 61.6% of total calories from carbohydrate, 24.8% of total calories from protein and 13.6% of total calories from fat) and in a second experiment (n=7) on a high fat diet (HFD) *ad libitum* (Research Diets, Inc., USA, D12451, 4.7 kcal/g, 35% of total calories from carbohydrate, 20% from protein and 45% from fat). After six weeks, saline (2 ml/kg) or porcine PYY\textsubscript{3-36} (300 µg/kg) were injected intraperitoneally (ip), 1h before the onset of the dark phase. In a third experiment, saline or PYY\textsubscript{3-36} were administered in SCD rats after 14 days of 50% food restriction (n=7). All experiments were performed in accordance with the Swiss regulations for animal experimentation.

*Transmitter Implantation.* Rats were anaesthetized by inhalation of isoflurane (2-3 vol% in medicinal oxygen). The operation area was shaved, sterilized with iodine and a 4-5 cm midline abdominal incision was made. The intestines were gently retracted with saline-soaked sterile gauze permitting access to the abdominal aorta from the renal arteries to the iliac bifurcation. The abdominal aorta was separated from the vena cava distal to the renal arteries. To occlude temporarily the abdominal aorta, a vascular clamp (Fine Science Tools GmbH, Germany) was used. While the aorta was clamped, the catheter of the telemetry transmitter (TL11M2-C50-PXT, Data Sciences International, USA) was inserted and secured with a single drop of tissue adhesive (Vetbond\textsuperscript{TM}). The vascular clamp was removed and the catheter entry site was checked for leakage. The transmitter was sutured to the inside surface of the peritoneum and the abdominal and skin incisions were closed with a non-absorbable suture (Ethicon\textsuperscript{®}, silk 3-0 and Prolene\textsuperscript{®} 4-0, respectively). All procedures were performed aseptically and according to the recommendations of the manufacturer (Data Sciences International, USA).

*Statistical Analysis.* Baseline values of MAP, HR, BT and LA under different dietary regimens were registered by telemetry for 10 seconds every 30 minutes over 24 hours. The time taken to change the cages and to determine body weight and food intake (usually 1 hour) was excluded from analysis. Thus 11-hour averages are shown for the light phase and 12-hour averages for the dark phase. The values given in Table 1 represent a single 23-h period.

A higher sampling rate (i.e. 10 seconds every 10 minutes over 24 hours) was chosen, when PYY3-36 or vehicle was injected. Telemetry data were analysed during 3 hours (18:00-21:00) (Figure 2, 3) with the One Way Repeated Measures ANOVA followed by Bonferroni t-test. Values are expressed as means ± SEM.

*Substances.* Porcine PYY\textsubscript{3-36} (MW 3980) was purchased from Neosystem (Strasbourg, France) and was dissolved in sterile saline immediately before ip injection.
Results

Effects of Different Dietary Regimens. Body weight increased from 390 ± 6 g to 491 ± 13 g (p<0.001) in ad libitum fed SCD rats during a 6-week period and was significantly reduced after 2 weeks of 50% food restriction (from 491 ± 13 g to 418 ± 8 g, p<0.001) (Fig.1). In ad libitum fed HFD rats body weight increased from 389 ± 4 g to 542 ± 12 g (p<0.001). Thus, an additional body weight increase of approximately 10 percent was observed in HFD rats compared to SCD fed controls. Daily food intake did not change throughout the experiment in SCD fed controls (average over 6 weeks: 86 ± 1 kcal/d). Rats on food restriction received 50 percent of their normal daily food intake (43 kcal/d). In rats on HFD a strong increase of caloric intake was observed after the rats received the HFD (from 84 ± 2 kcal/d on day 0 to 145 ± 9 kcal/d on day 1, p<0.001). This effect was not maintained, but the daily caloric intake over 6 weeks was still higher in rats on HFD compared to SCD fed controls (99 ± 4 kcal/d vs. 86 ± 1 kcal/d, p<0.01).

Baseline values of MAP, HR, BT and LA were significantly reduced after food restriction in comparison to SCD ad libitum (Tab.1). Changes of MAP, HR, BT and LA were more pronounced during the dark phase (-7 ± 1 mmHg, -86 ± 6 beats/min, -0.5 ± 0.1 °C and -0.5 ± 0.4 counts/min; food restriction vs. SCD ad libitum) than during the light phase (-2 ± 1 mmHg, -54 ± 5 beats/min, -0.5 ± 0.1 °C and -0.0 ± 0.2 counts/min; food restriction vs. SCD ad libitum). Overfeeding with a HFD for 6 weeks did not change baseline values of MAP, HR and BT compared to SCD fed controls, whereas LA tended to increase.

PYY3-36 and food intake. Peripheral administration of PYY3-36 (300 µg/kg, ip) resulted in a slight reduction of 24h-food intake in ad libitum fed SCD rats (79 ± 2 kcal/d vs. 88 ± 2 kcal/d in saline treated controls, p<0.05, paired t-test) and ad libitum fed HFD rats (91 ± 3 kcal/d vs. 102 ± 5 kcal/d in saline treated controls, p<0.05). There was no difference in the 24h-food intake in food restricted SCD rats after PYY3-36 administration since these rats always consumed all of the food that was offered to them.

Cardiovascular, temperature and locomotor responses to PYY3-36. Figure 2 shows the effects of PYY3-36 after 2 weeks of 50% food restriction, the group in which the strongest responses to PYY3-36 were observed. During the last hour before injection, values for MAP, HR, BT and LA were similar in the respective PYY3-36 and vehicle treated groups. Immediately after injection (17:00h) a strong transient rise in MAP, HR, BT and LA occurred in all rats, which disappeared within 1 hour (injection artefact). In the following stable period MAP, HR and BT were significantly elevated in PYY3-36 treated rats compared with vehicle treated controls. PYY3-36 increased MAP by 7 ± 1 mmHg during 3 hours after administration (p<0.001, One Way Repeated Measures ANOVA followed by Bonferroni t-test), HR by 22 ± 4 beats/min (p<0.001) and BT by 0.2 ± 0.0 °C (p<0.05)
(Fig.2). The cardiovascular and temperature responses occurred in the absence of any change in locomotor activity.

Figure 3 shows a summary of the cardiovascular, temperature and locomotor responses of PYY3-36 during different dietary regimens. In contrast to increased MAP, HR and BT in food restricted rats, PYY3-36 did not change MAP (0 ± 1 mmHg) and HR (-8 ± 5 beats/min) in rats on a HFD. Rats on a SCD ad libitum showed an intermediate response (MAP +4 ± 1 mmHg, p<0.05 and HR +5 ± 2 beats/min, not significant).

Discussion

Adaptation to a High Fat Diet and Food Restriction. Caloric intake was higher in HFD than in SCD rats (total difference: +546 kcal after 6 weeks), which explains the additional rise of body weight in HFD rats (+51g after 6 weeks, 1g fat=9 kcal) and suggests that metabolism was increased in HFD rats probably due to increased LA. Overfeeding with a high fat diet for 6 weeks did not change MAP and HR significantly compared with SCD fed controls. These results are consistent with a previous observation (16) that diets with an increased amount of fat do not significantly raise blood pressure and heart rate after 6 weeks in conscious, unrestrained normotensive rats and suggest that overfeeding with a high fat diet is only associated with a small stimulation of cardiovascular sympathetic tone. Overfeeding with a high fat diet did not change BT either. However, during the dark phase BT tended to decrease in HFD rats compared with SCD fed controls, which might be ascribed to the fact that lipids, a main constituent of the HFD, are less thermogenic than carbohydrates, a main constituent of the SCD (17). Most of the daily food intake occurs during the dark phase, which could explain, why the slightly reduced BT values were only observed during this period.

The significant decrease in body weight after 2 weeks of 50% food restriction was associated with strong reductions in MAP, HR, BT and LA. Suppression of the sympathetic nervous system is the most likely explanation (18). The observation that these changes were stronger during the dark phase than during the light phase probably reflects high sympathetic nerve activity during the dark phase and low sympathetic nerve activity during the light phase. The different effects of sympathetic withdrawal during the dark and light phase resulted in blunted circadian rhythms in food restricted rats.

PYY3-36 and Cardiovascular Regulation. The primary new finding of the present study is that Y2 receptor stimulation by PYY3-36 exerted cardiovascular effects in conscious, unrestrained normotensive rats and that the magnitude of these cardiovascular effects differed depending on the dietary regimen. In food restricted SCD rats, PYY3-36 increased MAP and HR, while in ad libitum fed HFD rats, PYY3-36 did not affect MAP and HR.
We assume that this difference corresponds to increased hypothalamic NPY release after food restriction and decreased hypothalamic NPY release during overfeeding with a HFD (5, 6).

The different pattern of PYY\textsubscript{3-36} in food restricted rats and rats on a HFD suggests opposite effects of central and peripheral NPY Y2 receptor stimulation. In food restricted rats (with high endogenous NPY tone) the central effects of NPY Y2 receptor stimulation dominated, which resulted in an increase of MAP and HR. Conversely, the peripheral effects of NPY Y2 receptor stimulation dominated in HFD rats (with low central NPY tone), which tended to decrease HR probably due to presynaptic inhibition of sympathetic neurotransmitters such as norepinephrine (19, 20, 21). The fact that blood pressure did not decrease after PYY\textsubscript{3-36} in HFD rats which probably had an increased sympathetic tone could be due to regionally selective vascular effects of Y2 receptor stimulation. It has been shown that blood pressure was hardly affected after peripheral administration of the Y2 receptor agonist NPY\textsubscript{13-36} in anaesthetized rats (22) while cutaneous microvascular blood flow substantially increased. This observation could explain, why PYY\textsubscript{3-36} injection induced a fall in body temperature in the HFD rats (probably a consequence of an increase in cutaneous blood flow) while blood pressure remained unchanged.

In the present experiments we administered PYY\textsubscript{3-36} systemically, because it has been demonstrated that after peripheral administration PYY\textsubscript{3-36} acts on the arcuate nucleus to stimulate presynaptic Y2 receptors (15). This is probably due to the fact that the blood brain barrier is absent or leaky in this brain region (1). The icv administration of PYY\textsubscript{3-36} does not appear to be a suitable approach to differentiate between the central and the peripheral cardiovascular actions of PYY\textsubscript{3-36}, because it may lead to a different pattern of NPY receptor activation in the brain. This assumption is based on the observation that icv administration of PYY\textsubscript{3-36} in mice increases food intake (23, 24), an effect opposite to that observed after peripheral administration (15). These divergent responses of food intake to PYY\textsubscript{3-36} are probably due to the fact that after icv administration PYY\textsubscript{3-36} penetrates more widely into the brain and may then act on Y2 receptors in other brain regions and also on Y5 receptors, for which PYY\textsubscript{3-36} exhibits a lower affinity (14). A different pattern of NPY receptor activation may not only elicit a different feeding response but also a different pattern of cardiovascular effects.

In standard chow diet fed controls MAP was slightly elevated without a change of HR, which is consistent with a previous observation that intracerebroventricular injection of the Y2-receptor preferring agonist NPY\textsubscript{13-36} produces an increase in blood pressure without effect on HR in conscious rats (25). Moreover, central NPY Y2 receptor stimulation via NPY\textsubscript{24-36} significantly increased blood pressure in anaesthetised dogs (26) and mean arterial pressure tended to be higher in Y2 receptor knockout mice (27).
The magnitude of the changes in MAP and HR observed in our studies might seem small but still be of clinical relevance. They are equal to those seen after food restriction or regular physical exercise in human subjects (28). Moreover, prospective clinical studies have demonstrated that even small blood pressure changes over a long period of time are associated with a considerable risk for cardiovascular diseases (29).

**PYY\textsubscript{3-36} and Body Temperature.** Icv administration of NPY or microinjection directly into the PVN have been shown to suppress the thermogenic sympathetic nerve activity (SNA) as demonstrated by direct measurement of the SNA to brown adipose tissue (30). Thus, administration of PYY\textsubscript{3-36}, which acts in the central nervous system to inhibit NPY release, should be associated with elevated thermogenic SNA and increased body temperature. In fact, we observed higher BT values after PYY\textsubscript{3-36} administration in our experiments, but only in food restricted rats, which have a high central NPY tone. In *ad libitum* fed SCD rats this stimulatory effect on BT was not seen.

NPY Y\textsubscript{1} and Y\textsubscript{2} receptors in the peripheral nervous system are also involved in thermoregulation, since they modulate vascular tone. It has been demonstrated that the activation of presynaptic Y2 receptors in the periphery produces cutaneous vasodilation due to presynaptic inhibition of norepinephrine at blood vessels of the skin and superseded the postsynaptic Y1 receptor mediated vasoconstriction (22). The subsequent heat loss evoked by cutaneous vasodilation might explain the decrease of BT observed in HFD rats after PYY\textsubscript{3-36} administration.

**PYY\textsubscript{3-36} and Locomotor Activity.** PYY\textsubscript{3-36} did not change LA in food restricted SCD rats, but reduced LA significantly in *ad libitum* fed SCD and HFD rats. These findings indicate that the locomotor response did not contribute to increased MAP, HR and BT observed in food restricted SCD rats after PYY\textsubscript{3-36} administration. Conversely, it may be that the locomotor activity after PYY\textsubscript{3-36} is related to the changes in food intake. Locomotor activity was reduced when food intake was lowered by PYY\textsubscript{3-36} administration in the *ad libitum* fed SCD and HFD groups while it remained unchanged in SCD rats on food restriction.

In summary, divergent cardiovascular responses to PYY\textsubscript{3-36} were observed in rats on different dietary regimens. These findings suggest that the cardiovascular effects of PYY\textsubscript{3-36} depend on the pre-existing hypothalamic NPY release and that central NPY has a tonic influence on blood pressure and heart rate, which becomes prominent during caloric deprivation.

**Acknowledgements**

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Reference


Figure 1. Influence of a standard chow diet ad libitum, 50% restriction of a standard chow diet and a high fat diet ad libitum on body weight and 24h-food intake in male Sprague-Dawley rats (n=7, each group). Values are means ± SEM. *p<0.05 vs. standard chow diet ad libitum.
Figure 2. Influence of ip injection of PYY\textsubscript{3-36} (300 µg/kg, black symbols) or vehicle (saline, 2 ml/kg, white symbols) on MAP, HR, BT and LA in SCD rats after 14 days of 50% food restriction. Values are means ± SEM. The arrows indicate the injection time (17:00h). Statistical evaluation of 3h-intervals (18:00-21:00h, horizontal line) was performed by using the One Way Repeated Measures ANOVA followed by Bonferroni t-test. *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\), ns (not significant): PYY\textsubscript{3-36} vs. saline.
Figure 3. Summary of the cardiovascular, temperature and locomotor responses of PYY\textsubscript{3-36} after different dietary regimens. The delta (\(\Delta\)) represents the difference between the response to vehicle and PYY\textsubscript{3-36} injections. After ip administration of PYY\textsubscript{3-36} (300 \(\mu\)g/kg) or vehicle (saline, 2 ml/kg) 3h-intervals (18:00-21:00h) were analysed by using the One Way Repeated Measures ANOVA followed by Bonferroni t-test. *\(p<0.05\), **\(p<0.01\), ***\(p<0.001\), ns (not significant): PYY\textsubscript{3-36} vs. saline.
Table 1. Baseline Values of Mean Arterial Pressure (MAP), Heart Rate (HR), Body Temperature (BT) and Locomotor Activity (LA) after Different Dietary Regimens

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<th>Standard Chow Diet</th>
<th>Standard Chow Diet</th>
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<td>50% Food Restriction</td>
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<td>ad libitum (6 weeks)</td>
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<td></td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
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<tr>
<td>MAP, mmHg</td>
<td>101±3</td>
<td>103±3&lt;sup&gt;T&lt;/sup&gt;</td>
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<tr>
<td>HR, beats/min</td>
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<td>294±4&lt;sup&gt;+++&lt;sub&gt;+++&lt;sub&gt;T&lt;/sub&gt;&lt;/sub&gt;&lt;/sup&gt;</td>
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<td>BT, °C</td>
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<td>37.7±0.1&lt;sup&gt;+++&lt;sub&gt;†&lt;sub&gt;T&lt;/sub&gt;&lt;/sub&gt;&lt;/sup&gt;</td>
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<td>LA, counts/min</td>
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Values are means ± SEM, n=7 (each group). One Way Repeated Measures ANOVA followed by Bonferroni t-test.

<sup>T</sup>p<0.05, <sup>†</sup>p<0.01, <sup>+++</sup>p<0.001: Food restriction (50%) vs. standard chow diet ad libitum.

<sup>†</sup>p<0.05, <sup>††</sup>p<0.01, <sup>+++</sup>p<0.001: Food restriction (50%) vs. high fat diet ad libitum.

<sup>†</sup>p<0.05, <sup>††</sup>p<0.01, <sup>+++</sup>p<0.001: High fat diet ad libitum vs. standard chow diet ad libitum.