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Peptide YY Directly Inhibits Ghrelin-Activated Neurons of the Arcuate Nucleus and Reverses Fasting-Induced c-Fos Expression

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Key Words

Electrophysiology · Immunohistochemistry · Food intake · Energy balance · Ghrelin · Brainstem · c-Fos · Arcuate nucleus · Peptide YY · Area postrema · Mice · Neurosteroids

Abstract

The hypothalamic arcuate nucleus (Arc) monitors and integrates hormonal and metabolic signals involved in the maintenance of energy homeostasis. The orexigenic peptide ghrelin is secreted from the stomach during negative status of energy intake and directly activates neurons of the medial arcuate nucleus (ArcM) in rats. In contrast to ghrelin, peptide YY (PYY) is released postprandially from the gut and reduces food intake when applied peripherally. Neurons in the ArcM express ghrelin receptors and neuropeptide Y receptors. Thus, PYY may inhibit feeding by acting on ghrelin-sensitive Arc neurons. Using extracellular recordings, we (1) characterized the effects of PYY on the electrical activity of ghrelin-sensitive neurons in the ArcM of rats. In order to correlate the effect of PYY on neuronal activity with the energy status, we (2) investigated the ability of PYY to reverse fasting-induced c-Fos expression in Arc neurons of mice. In addition, we (3) sought to confirm that PYY reduces food intake under our experimental conditions. Superfusion of PYY reversibly inhibited 94% of all ArcM neurons by a direct postsynaptic mechanism. The PYY-induced inhibition was dose-dependent and occurred at a threshold concentration of 10^{-8} *M*. Consistent with the opposite effects of ghrelin and PYY on food intake, a high percentage (50%) of Arc neurons was activated by ghrelin and inhibited by PYY. In line with this inhibitory action, peripherally injected PYY partly reversed the fasting-induced c-Fos expression in Arc neurons of mice. Similarly, refeeding of food-deprived mice reversed the fasting-induced activation in the Arc. Furthermore, peripherally injected PYY reduced food intake in 12-hour fasted mice. Thus the activity of Arc neurons correlated with the feeding status and was not only reduced by feeding but also by administration of PYY in non-refed mice. In conclusion, our current observations suggest that PYY may contribute to signaling a positive status of energy intake by inhibiting Arc neurons, which are activated under a negative status of energy intake by signals such as ghrelin.

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Introduction

Peptide YY (PYY), a 36-amino-acid peptide structurally related to neuropeptide Y (NPY) and pancreatic polypeptide (PP), was originally isolated from porcine intestine [1]. It is released from endocrine L cells of the ileum and colon [2, 3]. Plasma levels of PYY increase in response to food intake in different species including humans [4–6]. In addition to PYY, another biologically active molecular isoform, PYY(3–36), is physiologically released after a meal [7]. PYY has been proposed to be involved in the regulation of gastrointestinal function since it inhibits gastric acid secretion, gastric emptying, pancreatic secretion, intestinal secretion and gastrointestinal motility [8–12]. Although PYY is a potent stimulator of feeding when injected into the CNS (intracerebroventricular and intra-paraventricular application) [13–16], data on the effect of peripherally administered PYY on feeding behavior are scarce. However, an anorectic action of PYY(3–36) has been reported recently [17]. This anorectic effect appears to be mediated by the hypothalamic Arc, since intra-arcuate injection of PYY(3–36) also reduced food intake in these studies. Moreover, the inhibition of feeding induced by PYY(3–36) is dependent upon the Y2-receptor subtype. In line with its pharmacological profile, PYY(3–36) fails to inhibit feeding in Y2 receptors knockout mice [17]. PYY(3–36) specifically binds to Y2 receptors with high affinity [18], in contrast to PYY, which binds to at least three Y-receptor subtypes.

Different populations of neurons in the Arc contain the orexigenic peptide NPY and the anorectic pro-opiomelanocortin (POMC)-derived peptide α-melanocyte-stimulating hormone (α-MSH) [19]. In electrophysiological patch-clamp studies, PYY(3–36) indirectly activated POMC-expressing Arc neurons of mice by a presynaptic action [17]. The Y2 receptors, which appear to mediate this effect, are predominantly expressed in NPY neurons [20].

Peripherally released PYY may counterregulate the effects of the orexigenic hormone ghrelin on energy homeostasis. Ghrelin is a recently discovered 28-amino-acid peptide [21] secreted from the stomach during fasting [22]. It has been identified as an endogenous high-affinity ligand for the growth hormone secretagogue receptor (GHS-R), a G-protein-coupled receptor highly expressed in the Arc [23, 24]. Ghrelin stimulates food intake after both central and peripheral administration [25, 26]. As demonstrated by recent studies, ghrelin [27, 28] and artificial growth hormone secretagogues [29, 30], which also bind to the GHS-R, directly activate leptin-responsive cells of the ArcM, where NPY-expressing neurons are regionally clustered [20]. As an indirect effect, ghrelin presynaptically inhibits neurons in the lateral Arc (ArcL) [28], where POMC-expressing neurons predominate [20]. In mice, however, POMC- and NPY-containing neurons are equally distributed throughout the Arc.

Proceeding from the aforementioned observations it was the aim of our current investigation to analyze the effect of PYY on the neuronal activity of the primary target neurons for ghrelin, which at least in rats appear to be mainly located in the ArcM. Using an in vitro slice preparation of the Arc of rats, we performed extracellular single unit recordings to compare ghrelin- and PYY-induced effects at the single cell level. Since PYY reduced the activity of Arc neurons in rats, we also investigated the ability of PYY to reverse neuronal activation in the Arc of mice, which had been induced by food deprivation.

Similar to the Arc, the area postrema (AP) of the brainstem represents a receptive site for humoral factors regulating feeding behavior, such as the anorectic hormone amylin, which inhibits food intake by activating AP neurons [31, 32]. We recently demonstrated that neuronal activity of the AP is increased by feeding-related stimuli, especially by endogenously released amylin [33]. Peripheral administration of PYY has been shown to induce c-Fos expression in the AP [34], suggesting that PYY may also inhibit feeding via an activation of AP neurons. For this reason, we investigated whether PYY induces a c-Fos expression in the AP under our experimental conditions.

Finally, in order to correlate the effect of PYY on neuronal activity in the Arc with feeding behavior, we further determined whether peripherally injected PYY reduces food intake under the same conditions as in the immunohistological study.

Materials and Methods

Electrophysiological Studies

Male adult Wistar rats (200–250 g) were used, which had ad libitum access to standard laboratory rat chow (890 25 W16, Provimi Kliba AG, Gossau, Switzerland) and water. All animals were maintained in a temperature-controlled room on an artificial 12-hour dark-light cycle (21 ± 1 °C, lights on at 06:00 h).

The experimental procedures for the extracellular recordings were the same as previously described [28]. Rats were decapitated, and their brains were quickly removed and superfused with ice-cold artificial cerebrospinal fluid (aCSF) of the following composition (in m*M*): NaCl 124; KCl 5; NaH_2PO_4 1.2; $MgSO_4$ 1.3; $CaCl_2$ 1.2; $NaHCO_3$ 26; glucose 10, equilibrated with 95% O_2 and 5% CO_2, pH 7.4; 290 mosm/kg. 400-µm-thick coronal whole brain slices at the mid-rostrocaudal level of the Arc were cut using a custom-made vibratome. A rectangular (2 × 2 mm) piece of tissue containing the

Arc was dissected by hand and transferred to a temperature-controlled (37.0°C) recording chamber constantly superfused with prewarmed aCSF at a rate of 1.6 ml/min.

Extracellular recordings were obtained from neurons of the ArcM using glass-coated platinum-iridium electrodes. According to the neuroanatomical brain map of Paxinos and Watson [35], the recorded neurons were located within a distance of 300 µm lateral to the third ventricular wall and were thus defined as 'ventromedial' Arc neurons [see also 28]. After a stable recording from a single neuron had been established, its responsiveness to ghrelin and PYY (both from Bachem, Switzerland) was tested by switching to a solution containing the investigated peptide at a concentration of 10^{-8} *M* and 10^{-9}– 10^{-7} *M*, respectively. In dose-response investigations of a preceding study [27], a ghrelin concentration of 10^{-8} *M* has been demonstrated to represent an effective dose, which did not cause a desensitization of the involved receptor. In order to exclude that the superfusion of the vehicle that was used to dissolve the peptides causes changes in the firing rate of Arc neurons, control superfusions with vehicle were carried out. From the continuously recorded rate meter counts, the average discharge rate of each neuron was evaluated for 60 s prior to the stimulus. This value (spontaneous discharge rate) was used to normalize changes in firing rate, expressed as % change of the spontaneous discharge rate. If the averaged change of discharge rate during the response was larger than ±20%, the neuron was considered sensitive to the applied substance. In addition to the mean change of the discharge rate during the entire responses, the peak values of the responses were calculated on a basis of a 30-second interval during which the firing rate was maximal or minimal, respectively. Finally, the duration between the application of the drugs and the onset of the effects (latency) and the duration of the entire responses were determined. The parameters of the electrophysiological responses were expressed as means ± SE.

Similar to the experimental approach of our recent study [28], it was tested whether the PYY-induced effects on neuronal activity in the Arc depended on synaptic interactions. Therefore, the responses to PYY were investigated using aCSF containing a low Ca^{2+} (0.3 m*M*) and a high Mg^{2+} (9 m*M*) concentration, which is known to block synaptic transmission [36].

c-Fos Studies

Male mice (C57BL/6N), weighing 24–31 g, were randomly assigned to one of three experimental groups (4–5 animals each). The animals were housed individually in wire cages in a temperature-controlled room under a 12-hour artificial light cycle. Standard rodent pellets (890 25 W16, Provimi Kliba AG) were fed and ad libitum access to water was provided. The animals were handled daily from at least 1 week prior to the experiment. Mice were food-deprived during the light phase. At dark onset, two groups of animals received either a subcutaneous injection of PYY (50 µg/kg) or saline. The third group was given access to food. 120 min after the injections or refeeding, the mice were anesthetized with pentobarbital (Nembutal, Abbott Laboratories, USA; 80 mg/kg i.p.) and perfused transcardially with ice-cold phosphate buffer (PB 0.1 *M*), followed by 4% paraformaldehyde (in 0.1 *M* PB). The brains were removed and kept in 4% paraformaldehyde for additional 2 h. After 48 h of incubation in 20% sucrose solution at 4°C (in 0.1 *M* PB) for cryoprotection, the samples were snap-frozen in CO_2 gas. Coronal sections, 20 µm thick, were cut in a cryostat (CM 3050, Leica, Nussloch, Germany) throughout the hypothalamic area of the Arc and the AP. Every section was thaw-mounted on microscopic glass slides (SuperFrost Plus, Faust, Schaffhausen, Switzerland) and stored at –20°C until further processing. For the detection of c-Fos expression the immunoperoxidase procedure was employed as follows. The sections were air-dried at room temperature for 1 h and rehydrated in 0.1% PBS (phosphate-buffered saline solution, 2 × 5 min). This washing procedure was repeated after a 30-min incubation in PBS containing 0.3% H_2O_2. Unspecific binding was blocked with a 2-hour incubation in 1.5% normal rabbit serum. After washing, the primary antibody (1:10,000 polyclonal goat anti-c-Fos, Santa Cruz Biotechnology, Calif., USA) was applied for 48 h at 4°C. The unbound antibody was removed by washing in PBS and the sections were incubated with the secondary antibody (1:200 biotinylated rabbit-anti-goat, Vectastain-Elite ABC Kit, Vector Laboratories, Burlingame, Calif., USA) for 2 h at room temperature. c-Fos immunoreactivity (c-Fos-IR) was detected by incubation in diaminobenzidine (DAB) solution (0.04% in PBS with 0.02% H_2O_2, 0.08% $NiCl_2$, 0.01% $CoCl_2$). The sections were dehydrated in graded alcohol, incubated in xylol and coverslipped. The localization of the c-Fos-expressing neurons was identified according to the mouse brain atlas by Hof et al. [37]. In 21 sections of the Arc between bregma –1.10 and –2.40 and 7 sections of the AP from corresponding levels in each animal, the numbers of c-Fos-IR neurons were quantified by using a light microscope (Axioscop 2, Carl Zeiss AG, Feldbach, Switzerland). In each group the mean value of the cell counts/section of an individual animal was used for statistical analysis (n = number of animals). Photomicrographs were taken by a digital camera (AxioCam, Carl Zeiss AG).

Results are presented as means ± SE. For comparison of more than two treatment groups, a one-way analysis of variance (ANOVA) was used, followed by Student-Newman-Keuls post-hoc test (quantification of c-Fos-IR neurons in the Arc). For statistical analysis of c-Fos expression in the AP of PYY-treated vs. saline-injected mice, the unpaired t test was applied. Differences between the groups were considered significant with $p < 0.05$ for all statistical tests.

Feeding Studies

Feeding studies with a crossover design were conducted with 10 male C57BL/6N mice (body weight 24–27 g). The housing and handling conditions corresponded to those described for the c-Fos studies. Animals were food-deprived for 12 h during the light phase. At dark onset, vehicle (saline) or PYY (50 µg/kg) was administered subcutaneously and food was offered immediately after injection. Food intake was determined by weighing of the food cups after 1 and 2 h. Paired t test was performed to compare the effects of the treatments.

Results

Electrophysiological Studies

In total, 69 single unit recordings from neurons located in the ArcM were obtained from 45 slice preparations. In 28 recordings, the co-sensitivity of ghrelin and PYY was tested by successive superfusion of these peptides. The basal discharge rate under unstimulated control conditions ranged between 1.4 and 12.5 Hz.

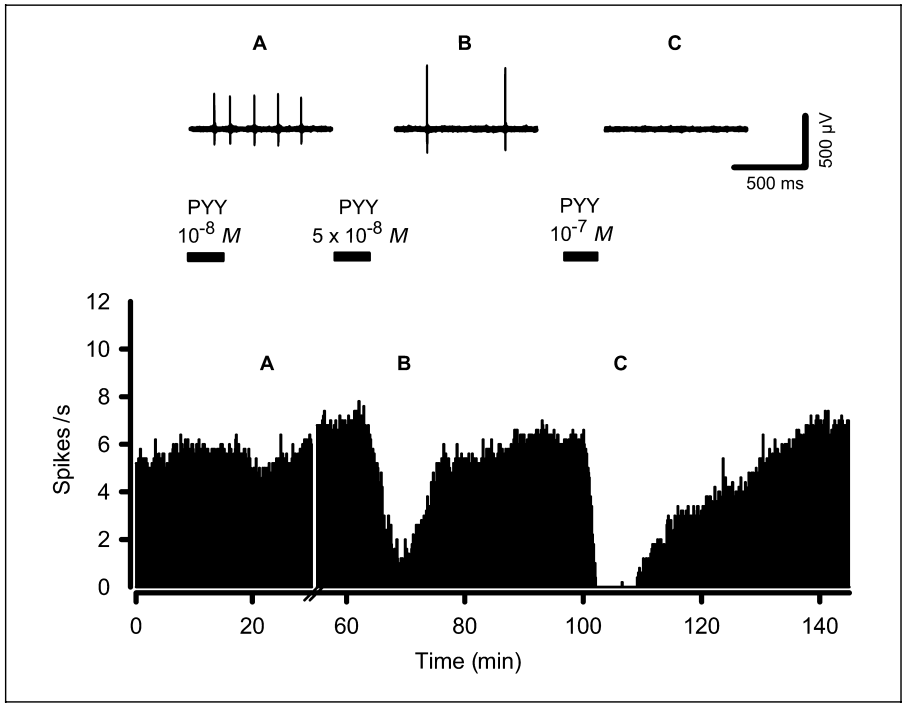

Fig. 1. Continuous rate meter recording of a spontaneously active neuron from the medial arcuate nucleus (ArcM) of the rat. Consecutive superfusions of PYY at different concentrations during the indicated times caused dose-dependent inhibitory effects. Representative segments of the original recording of action potentials at the indicated times during the effects (**A–C**) are displayed in the upper trace.

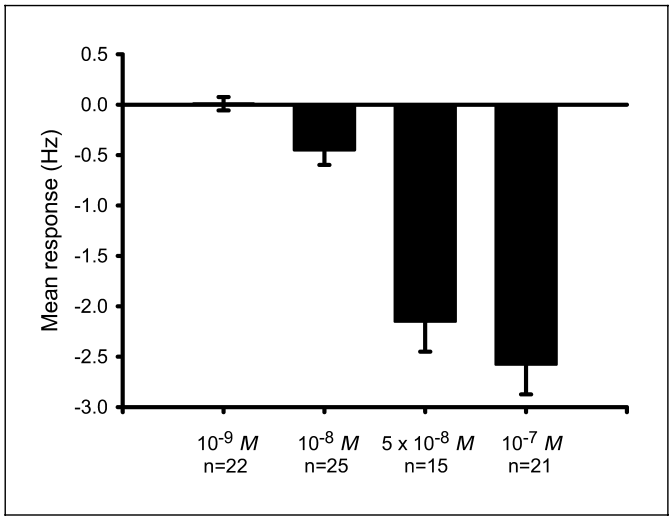

Fig. 2. Averaged dose dependency of the PYY-induced inhibitory effect in the medial arcuate nucleus (ArcM) of rats. Superfusion of PYY at a concentration of 10^{-9} *M* did not produce any changes in the firing rate. The mean threshold concentration for the inhibitory action of PYY was 10^{-8} *M*.

Superfusion with PYY (10^{-8}–10^{-7} *M*) inhibited 94% of all cells tested with this peptide (n = 69). Excitatory effects were not observed, and the remaining 6% of cells were insensitive. Control superfusions of vehicle caused no changes in the firing rate (n = 13). All inhibitory responses mediated by PYY were reversible. As shown by a representative recording (fig. 1), the PYY-induced inhibition was dose-dependent. Segments of the original spike recording taken during the responses of each PYY application (top trace fig. 1) illustrate that signals could be easily discriminated from noise due to a high signal to background ratio (on average 9:1 for all recordings in this study). As shown by the averaged dose-response relationship (fig. 2), the threshold concentration for the inhibitory PYY action was 10^{-8} *M*. The inhibitory effects induced by PYY at a concentration of 10^{-7} *M* were characterized by a short onset (171 ± 16 s, n = 59) and a long duration (1,331 ± 59 s). Additional effect parameters of all PYY-mediated inhibitions are summarized in table 1.

In order to determine whether the inhibitory action of PYY depends on synaptic interactions, 9 neurons which had been confirmed to be inhibited by PYY, were tested during synaptic blockade (low Ca^{2+}/high Mg^{2+} solution). In all recordings, the inhibitory effect of PYY persisted under low Ca^{2+}/high Mg^{2+} conditions, indicating that the observed inhibitions were direct postsynaptic effects on

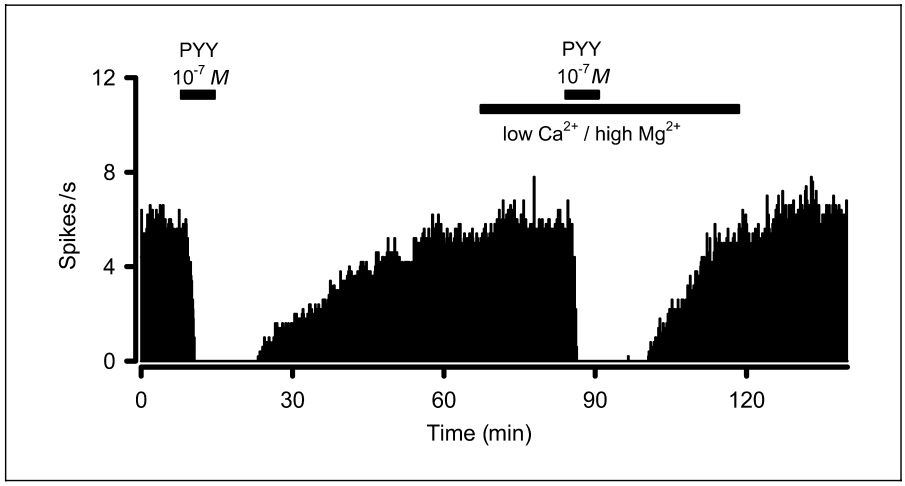

Fig. 3. Recording from a neuron of the rat ArcM showing that the inhibitory effect induced by PYY persisted in the presence of a solution containing a low Ca^{2+} concentration (0.3 m*M*) and a high Mg^{2+} concentration (9 m*M*), which blocks synaptic transmission. Both under standard conditions and during synaptic blockade, the neuron was effectively and reversibly inhibited by PYY, indicating that the inhibitory action induced by PYY is a direct postsynaptic effect.

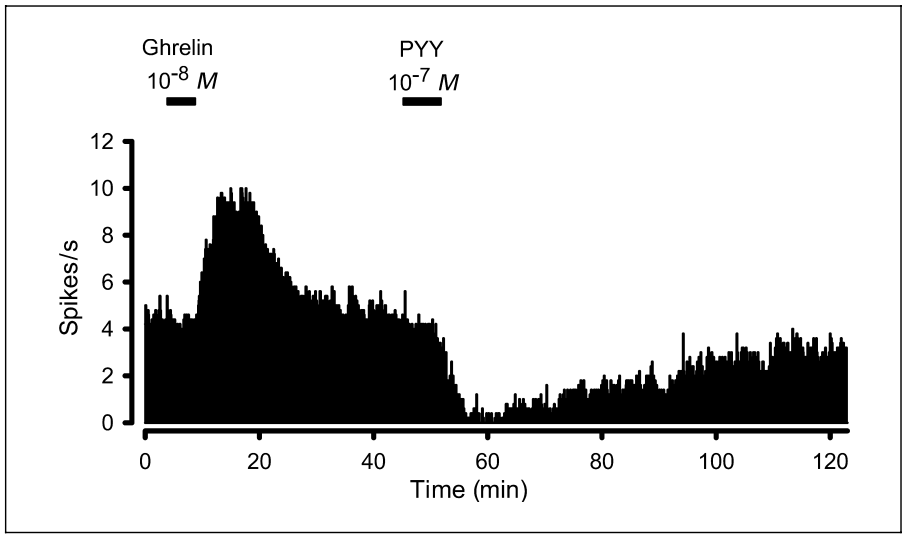

Fig. 4. Continuous rate meter recording of a spontaneously active neuron from the medial arcuate nucleus (ArcM) of the rat. Consecutive superfusions of ghrelin and PYY at the indicated times caused opposite effects on neuronal activity. While ghrelin induced a strong excitatory response, PYY effectively decreased the discharge rate.

the recorded neurons (fig. 3). Consistent with earlier studies [28, 38], most neurons (5 of 9) decreased their discharge rate during synaptic blockade from 4.4 ± 0.8 Hz by 1.8 ± 0.7 Hz. This decrease was most likely due to a blockade of excitatory glutamatergic neurotransmission [39]. Two neurons increased their firing rate and 2 cells showed no response during the superfusion of low Ca^{2+}/high Mg^{2+} solution.

In our current investigations we were able to reproduce our recent observation that neurons of the ArcM are predominantly excited by superfusion of ghrelin [28]. Under identical experimental conditions, 61% of all neurons tested with ghrelin (n = 28; for effect parameters see table 1) showed excitatory responses, only 7% were inhibited and the remaining cells were insensitive. There was a high degree of co-sensitivity to ghrelin and PYY (fig. 4).

Table 1. Effect parameters of the electrophysiological responses induced by PYY (10^{-7} *M*, n = 59) and ghrelin (10^{-8} *M*, n = 17); values are given as mean ± SE

Effect parameter	PYY inhibitory (n = 59)	Ghrelin excitatory (n = 17)
Mean spontaneous activity, Hz	4.1 ± 0.3	3.7 ± 0.3
Mean latency, s	171 ± 16	183 ± 27
Mean response, %	–68 ± 3	57 ± 10
Mean response, Hz	–2.6 ± 0.2	1.7 ± 0.1
Mean peak response, Hz	–3.3 ± 0.3	2.5 ± 0.5
Mean response duration, s	1,331 ± 59	1,078 ± 70

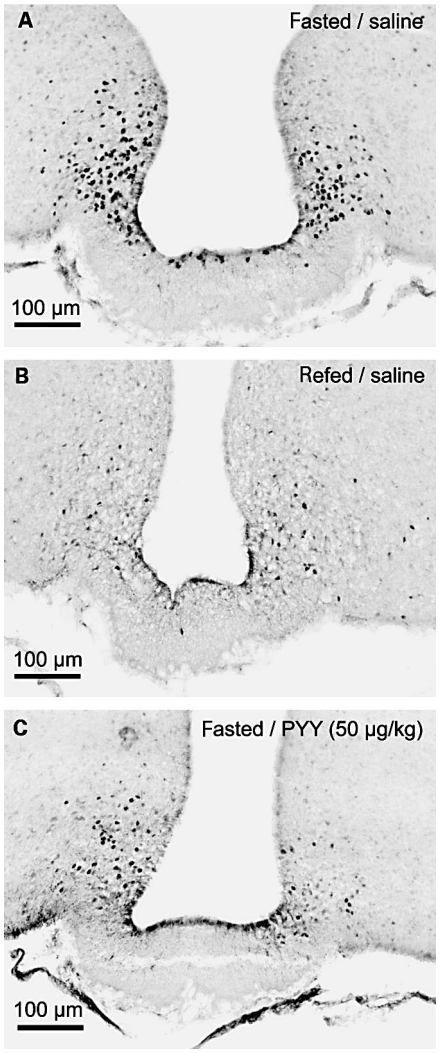

Fig. 5. Representative immunohistochemical c-Fos staining of 20-µm-thick coronal sections of the arcuate nucleus (mice). **A** 12-hour food deprivation elicited a strong c-Fos expression in the Arc. **B** The fasting-induced c-Fos expression was completely reversed by refeeding. **C** Subcutaneous injection of PYY (50 µg/kg) in non-refed mice partly reversed the fasting induced activation of Arc neurons.

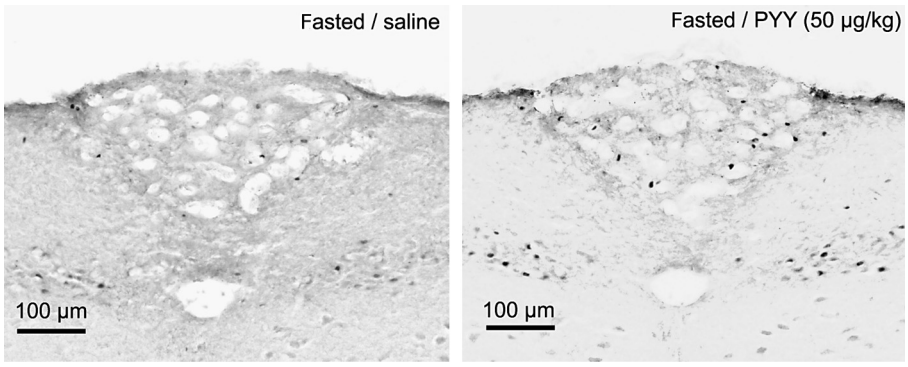

Fig. 6. Representative immunohistochemical c-Fos stainings of 20-µm-thick coronal sections of the area postrema (mice). Both in fasted/saline-treated mice and in fasted/PYY-injected animals (50 µg/kg, s.c.) the number of c-Fos-IR nuclei was low.

Table 2. Numbers of ArcM neurons responsive to PYY (10^{-7} *M*) and ghrelin (10^{-8} *M*); percentage of total cell numbers tested with both substances are given in parentheses

		Ghrelin			
		excited	inhibited	insensitive	total
PYY	excited	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	inhibited	14 (50%)	2 (7%)	8 (29%)	24 (86%)
	insensitive	3 (11%)	0 (0%)	1 (4%)	4 (14%)
	total	17 (61%)	2 (7%)	9 (32%)	28 (100%)

In 50% of the cells ghrelin produced excitatory effects, whereas PYY inhibited neuronal activity. Only 11% of all neurons were activated by ghrelin but did not respond to PYY (table 2). Since the excitatory action of ghrelin in the ArcM has already been shown to be a direct postsynaptic effect [28], experiments testing ghrelin under low Ca^{2+}/high Mg^{2+} conditions were not repeated in this study.

c-Fos Studies

Food deprivation induced a strong c-Fos expression in the Arc of the vehicle-treated control mice (40 ± 6 cells/section, n = 5), while hardly any c-Fos-positive nuclei were detectable in the Arc of the refed group (2 ± 1 cells/section). The fasting-induced activation of Arc neurons was not only reversed by refeeding but also by PYY administration (fig. 5). Although the number of c-Fos-positive Arc neurons was not significantly different between the fasted/PYY and the fasted/refed group, the latter mice showed the lowest number of c-Fos-IR cells (refed: 2 ± 1 vs. PYY: 16 ± 2, n = 4 for both groups). However, both groups displayed significantly fewer c-Fos-expressing nuclei than the fasted/saline group (ANOVA, followed by Student-Newman-Keuls post-hoc test, $p < 0.001$).

In the AP, PYY did not induce a c-Fos expression under conditions where it attenuated the fasting-induced activation of Arc neurons (fig. 6). In both saline and PYY-treated groups the average number of c-Fos-positive neurons was low and did not differ significantly (fasted/saline: 4 ± 1 vs. fasted/PYY: 5 ± 1, n = 4 for both groups).

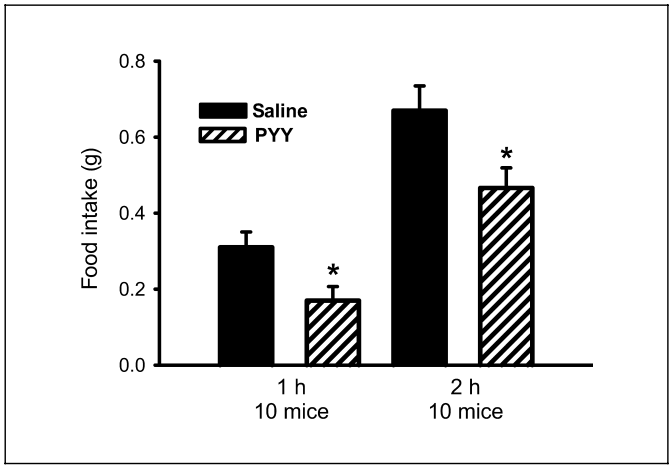

Fig. 7. Cumulative food intake of 12-hour food-deprived mice after subcutaneous injection of saline or PYY (50 μg/kg). PYY significantly reduced 1- and 2-hour food intake (paired t-test, * $p < 0.05$). Bars represent group means (mean ± SE).

Feeding Studies

The ability of PYY to reduce food intake was investigated under experimental conditions which were identical to those of the c-Fos studies. PYY significantly reduced 1- and 2-hour food intake compared to saline-treated controls. Cumulative food intake under control conditions (fasted/saline) was 0.31 ± 0.04 g (1 h) and 0.67 ± 0.07 g (2 h). In response to PYY administration, food consumption was significantly reduced by 45% after 1 h and 30% after 2 h compared to the saline-treated group (fig. 7).

Discussion

The Arc is considered an important receptive structure for circulating factors involved in the hypothalamic control of appetite and energy homeostasis. As suggested by previous studies, the orexigenic hormone ghrelin may counteract leptin-mediated effects in the Arc since it directly (postsynaptically) excites Arc neurons which are inhibited by leptin [27, 28]. In contrast to ghrelin, which is secreted during the absence of food and especially shortly before meal onset, the release of PYY from the gut is nutrient-dependent and increases in the postprandial state. Both in rats and humans, plasma levels of PYY rise within 30 min after a meal [40, 41].

The present study revealed dose-dependent inhibitory effects of PYY on the activity of neurons located in the ArcM of rats. This effect was mediated by a direct postsynaptic mechanism. In addition, PYY-inhibited ArcM neurons were excited by ghrelin.

The threshold concentration of the PYY-induced inhibition was 10^{-8} *M* under our in vitro conditions. Although this concentration is supraphysiological, the effective concentration for endogenously released PYY may be lower in vivo because of additive and/or synergistic effects with other humoral and/or neuronal stimuli associated with food intake. As such, the gut peptide glucagon-like peptide 1 (GLP-1) is co-released with PYY from the intestinal L-cells [40] but may also be locally released in the Arc from terminals originating from GLP-1 containing enteroceptive neurons located in the brainstem [42, 43]. Feeding studies employing a combined treatment with PYY(3–36) and GLP-1 suggest a concerted anorectic action of these peptides since they effectively reduce feeding when co-applied at subthreshold doses [44]. However, whether PYY-induced effects on the electrical activity in the Arc synergize with other feeding-related stimuli has not yet been investigated.

Full-length PYY binds to at least three Y-receptor subtypes (Y1, Y2 and Y5) [18, 45]. Only the Y2 receptor is densely expressed in the ArcM of rats. As shown by double-labeling studies, 92% of all NPY-immunoreactive neurons of the ArcM co-express the Y2 receptor [20]. Since the PYY-mediated inhibition of ArcM neurons observed in our study is a direct (postsynaptic) action, it is conceivable that the receptor mediating the inhibitory action of PYY in the ArcM is most likely the Y2 subtype.

Electrophysiological studies in mice demonstrated that Y2 receptor activation by PYY(3–36) indirectly (presynaptically) activates phenotypically identified POMC neurons in the Arc [17]. The effect of PYY on neuronal activitiy in the ArcL, where most of the POMC-expressing neurons are located in rats, has not been investigated in our study. However, PYY may likewise contribute to a presynaptic activation of POMC neurons in rats, since it also activates Y2 receptors, which mediate the indirect excitatory effect of PYY(3–36) on these cells in mice. These considerations suggest a dual mode of action for PYY on neuronal activity in the Arc. Neurons of the ArcM, which appear to express NPY, are directly inhibited by PYY, while ArcL neurons expressing the anorectic α-MSH may be indirectly excited, possibly by a reduction of inhibitory neurotransmission (disinhibition). Interestingly, at identical sites we could demonstrate an action of ghrelin opposite to that of PYY and PYY(3–36), consisting of a direct excitatory effect in the ArcM and an indirect inhibitory action in the ArcL [28]. Therefore, our current demonstration that ghrelin-activated neurons of the ArcM are

inhibited by PYY supports the idea that both peptides participate in signaling a negative and positive status of energy intake, respectively, by affecting neuronal activity in opposite ways.

In order to prove the concept that PYY inhibits Arc neurons which are activated under a negative status of energy intake, we investigated whether PYY reverses fasting induced c-Fos expression in the Arc of mice. In a similar experimental approach we successfully demonstrated that fasting induced c-Fos expression in the lateral hypothalamic area was reversed by peripheral injection of the anorectic hormone amylin [33]. For our current experiments we used mice which, in contrast to rats [unpubl. observations], express c-Fos in the Arc after food deprivation, while minimal activation is observed in ad libitum fed controls [46–48]. Our data demonstrating that refeeding reverses c-Fos expression in the Arc of food-deprived mice validates the assumption that neuronal activity in the Arc correlates with the feeding status. Peripheral injection of PYY in non-refed mice mimicked the effect of refeeding. This suggests that endogenously released PYY may contribute to an inhibition of Arc neurons. As discussed above, the inhibitory potency of endogenous PYY may be increased by other feeding-related stimuli, which may be only basally active or even inhibited under the fasting conditions of our experimental design. Consequently, our results rather underestimate than overestimate the impact that endogenously secreted PYY might exert on Arc neurons.

Our electrophysiological studies clearly show that PYY postsynaptically inhibits neurons of the ArcM, suggesting that the suppressive effects on c-Fos expression and food intake are mediated by a direct action of PYY on receptive neurons of the Arc. Involvement of Y2 rather than Y1 receptor is also suggested by another study using Y1 antagonists [49]. As evidenced by tracing studies, neurons in the Arc are accessible to blood-borne substances [50]. Moreover, a nonsaturable transport of PYY(3–36) across the blood-brain barrier has been reported in a recent study [51]. We thus propose that PYY directly targets Arc neurons to bring about the observed effects. However, these considerations do not rule out the possibility of indirect effects of PYY on Arc neurons mediated by other brain structures.

At least some of the gastrointestinal effects induced by PYY may be mediated by direct actions in the dorsal vagal complex [52–54], which contains the AP with an open blood-brain barrier. Peripherally injected radiolabeled PYY binds to Y receptors in the dorsal vagal complex [55] and a high density of Y receptors is present in the AP [56]. While an activation of AP neurons by feeding-related stimuli, such as the pancreatic hormone amylin, is considered as a neurophysiological correlate of satiety [31, 32], there are no indications that inhibitory signals in the AP may likewise induce satiety. Although peripheral PYY has been shown to induce c-Fos in the AP when injected at high doses (50–400 μg/kg) in rats [34], we could not detect a significant c-Fos response in mice under our experimental conditions. It thus appears unlikely that PYY physiologically inhibits feeding via an AP-dependent mechanism that would resemble that of the well-investigated hormone amylin [31, 32].

Anorectic factors (e.g. amylin, cholecystokinin (CKK), and gastric distension) acting via the brainstem to reduce food intake typically activate an ascending pathway including the nucleus of the solitary tract, the lateral parabrachial nucleus and the central nucleus of the amygdala. At least for CCK it has been shown that fasting induced c-Fos expression in the Arc of mice is not reversed by peripheral injection of CCK at doses which actually induce c-Fos in these areas [46]. Hence, functional evidence that activation of brainstem feeding pathways coincides with an inhibitory input on Arc neurons is still missing.

In summary, we conclude that postprandially released PYY may contribute to a direct inhibition of Arc neurons which are activated under negative status of energy intake. Since PYY-inhibited cells are activated by ghrelin, circulating PYY may promote satiety by opposing the effects of orexigenic factors acting via the Arc. The determinants contributing physiologically to an activation of Arc neurons under energy restriction and the question whether PYY acts in concert with other feeding-related stimuli remain to be investigated.

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