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Agonists for neuropeptide Y receptors Y_1 and Y_5 stimulate different phases of feeding in guinea pigs

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1 The stimulatory effect of neuropeptide Y (NPY) on food intake is well established but the roles of the receptor subtypes Y_1 and Y_5 have been difficult to define. We have studied the effects of two novel Y_1-preferring and two Y_5-preferring agonists on feeding in guinea pigs.

2 The Y_1-preferring receptor agonists [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY had high affinity for the Y_1 receptor (K_i values 0.07 and 0.04 nM, respectively) and nanomolar affinity for the Y_5 receptor. Administration of either compound into the third brain ventricle increased food intake equally to NPY.

3 The Y_5 agonist [Ala^{31},Aib^{32}]pNPY displayed a moderate affinity for the Y_5 receptor (K_i 7.42 nM) and a low affinity for Y_1 (K_i 1.7 μM). This compound had only a modest effect on feeding.

4 The other Y_5-preferring peptide [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP had a higher affinity at the Y_5 receptor (K_i 1.32 nM) and also at the Y_1 receptor (K_i 85 nM). It potently stimulated feeding: the food consumption after administration of this peptide was two-fold compared to NPY.

5 Our results support the view that both the receptor subtypes Y_1 and Y_5 are involved in the stimulation of feeding. As the action profiles of the Y_1 and Y_5 agonists on feeding parameters were different, it seems that they influence different phases of eating.

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Keywords: Neuropeptide Y; Y_1 receptor; Y_5 receptor; food intake; guinea pig

Abbreviations: hPP, human PP; NPY, neuropeptide Y; pNPY, porcine NPY; PP, pancreatic polypeptide; pPYY, porcine PYY; PYY, peptide YY

Introduction

Neuropeptide Y (NPY) is a 36 amino-acid peptide that belongs to the family of peptides that includes also pancreatic polypeptide (PP) and peptide YY (PYY). NPY is best known for its actions on feeding behaviour. When NPY or its peptide analogues are injected into the cerebral ventricles or directly into certain areas of the hypothalamus, feeding is increased (Stanley & Leibowitz, 1985; Stanley & Thomas, 1993; Haynes *et al*., 1998). The feeding elicited after NPY infusion has mostly been investigated in mice and rats (Levine & Morley, 1984; Morley *et al*., 1987), but has been observed in virtually all vertebrates that have been studied (Parrot *et al*., 1986; Kuenzel *et al*., 1987; Pau *et al*., 1988; Kulkosky *et al*., 1989; Miner *et al*., 1989; Morris & Crews, 1990; Boswell *et al*., 1993; Richardson *et al*., 1995; Larsen *et al*., 1999; Volkoff & Peter, 2001; Lecklin *et al*., 2002).

The receptor subtype mediating the effect of NPY on feeding has been difficult to define. First it was suggested that NPY induces feeding *via* Y_1 receptors (Kalra *et al*., 1991a; Leibowitz & Alexander, 1991). Later the Y_5 receptor was cloned and announced as a 'feeding' receptor (Gerald *et al*., 1996; Hu *et al*., 1996). The evidence to date suggests that both of these receptor subtypes, in addition to the presynaptic Y_2 receptor, are involved in food intake regulation in mice and rats (Criscione *et al*., 1998; Haynes *et al*., 1998; Kushi *et al*., 1998; Marsh *et al*., 1998; Pedrazzini *et al*., 1998; Wieland *et al*., 1998; Duhault *et al*., 2000; Polidori *et al*., 2000; Kanatani *et al*., 2001; Batterham *et al*., 2002; Lecklin *et al*., 2002).

Selective receptor ligands are useful tools to study the functions of a single receptor subtype. Selective nonpeptide antagonists have been described both for Y_1 and Y_5 receptors (Dumont *et al*., 2000), but a limitation with the use of NPY and its analogues is their poor receptor selectivity. Very recently, several agonists preferring either the Y_1 (Mullins *et al*., 2001; Söll *et al*., 2001) or the Y_5 receptor (Wyss *et al*., 1998; Cabrele *et al*., 2000; McCrea *et al*., 2000; Parker *et al*., 2000) have been identified. The purpose of the present study was to examine the contribution of Y_1 and Y_5 receptors to the control of food intake by studying the effects of two novel Y_1 agonists, [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY (Söll *et al*., 2001), and two Y_5 agonists, [Ala^{31},Aib^{32}]pNPY and [cPP$^{1-7}$, NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP (Cabrele *et al*., 2000), on feeding behaviour in guinea pigs. As NPY has been reported to influence the appetitive phase of feeding, the period before eating when the animal is looking for food, rather than the consummatory phase of feeding, the period of biting and

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swallowing (Woods *et al*., 1998; Ammar *et al*., 2000; Chamorro *et al*., 2002), special attention was paid to feeding parameters associated with appetitive and consummatory phases.

The guinea pig (*Cavia porcellus*) is a particularly useful animal model for NPY studies since its NPY receptors, in contrast to some of the rat and mouse receptors, show virtually identical pharmacological profiles to their human orthologues (Eriksson *et al*., 1998; Sharma *et al*., 1998; Berglund *et al*., 1999; Starbäck *et al*., 2000; Lundell *et al*., 2001). The guinea pig is very distantly related to rat and mouse (D'Erchia *et al*., 1996), thereby broadening the perspective on feeding behaviour in mammals. Furthermore, the guinea pig is a day-active animal allowing food intake measurements during the light phase. We have recently shown that central administration of NPY dose-dependently stimulates food intake in guinea pigs, and that the blockade of the Y_1 by selective antagonist attenuates the response to NPY (Lecklin *et al*., 2002). Here, we extended these studies by presenting results with agonists that confirm contributions from both Y_1 and Y_5 to food intake and that suggest partially distinct roles for the receptor subtypes in the stimulation of feeding.

Methods

Compounds

pNPY (porcine) was purchased from Bachem (King of Prussia, PA, USA). [Arg^6,Pro^{34}]pNPY, [Phe^7,Pro^{34}]pNPY, [Ala^{31}, Aib^{32}]pNPY and [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP were synthesised as described recently (Cabrele *et al*., 2000; Söll *et al*., 2001). Aib stands for alpha-aminoisobutyric acid. All compounds were dissolved in 0.9% saline.

Radioligand binding assays

Cell lines transfected with plasmids encoding the guinea pig receptors were used to study the selectivity and the affinities of the ligands as described earlier (Sharma *et al*., 1998; Berglund *et al*., 1999; Lundell *et al*., 2001). Inhibition experiments were carried out at concentration ranges for the radioligand of 0.022–0.040 nM for the guinea pig Y_1 receptor, 0.014–0.024 nM for the Y_2 receptor and 0.022–0.082 nM for the Y_5 receptor. The K_d values for the guinea pig Y_1, Y_2 and Y_5 receptors were 0.037, 0.006 and 0.410 nM, respectively (Sharma *et al*., 1998; Berglund *et al*., 1999; Lundell *et al*., 2001). For binding assays at Y_1, Y_2 and Y_5 receptors, thawed aliquots of receptor membranes were resuspended in 25 mM HEPES buffer (pH 7.4) containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 2 g l^{-1} bacitracin (Sigma, St Louis, MO, U.S.A.) and homogenised using an Ultra-Turrax homogeniser. Binding experiments were performed in a final volume of 100 μl with 2–10 μg protein and ^{125}I-pPYY (Amersham Pharmacia Biotech) for 2 h at room temperature. Nonspecific binding was defined as the amount of radioactivity remaining bound to the cell homogenate after incubation in the presence of 100 nM unlabelled pNPY. In competition studies, various concentrations of the compounds [Arg^6,Pro^{34}]pNPY, [Phe^7,Pro^{34}]pNPY, [Ala^{31},Aib^{32}]pNPY and [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP were included in the incubation mixture along with ^{125}I-pPYY. The peptide pNPY was used as a reference for each experiment. Incubations were terminated by rapid filtration through GF/C filters, which had been presoaked in 0.3% polyethyleneimine, using a TOMTEC (Orange, CT, U.S.A.) cell harvester. The filters were washed with 5 ml of 50 mM Tris (pH 7.4) at 4°C and dried at 60°C. The dried filters were treated with MeltiLex A (Wallac) melt-on scintillator sheets, and the radioactivity retained on the filters counted using the Wallac 1450 Betaplate counter. The results were analysed using the Prism software package (Graphpad, Dan Diego, CA, U.S.A.).

Animals

The study was approved by the local ethical committee (C121/00). Juvenile male Dunkin–Hartley guinea pigs (Bio Jet Service, Uppsala, Sweden) weighing 300–500 g were maintained in a 12-h light–dark cycle (lights on from 0600 to 1800 h) in a temperature-controlled room (20–21°C). Two animals were housed in a polypropylene cage (60 × 80 × 25 cm) and kept separated by dividing each cage into two equal parts by a wall. Throughout the experiment, guinea pigs were fed with powdered food (K5, Lactamin AB, Vadstena, Sweden) and hay was also freely available except during the tests. Tap water was supplemented with 0.5 mg ml^{-1}of L-ascorbic acid and was freely available. The feeding experiments were performed between 0930 and 1800.

Surgical procedures

Guinea pigs were anaesthesised by intraperitoneal (i.p.) injection of a 1 : 3 (v v^{-1}) mixture of xylazine (Rompun vet. 20 mg ml^{-1}, Bayer, Gothenberg, Sweden) and ketamine (Ketalar 50 mg ml^{-1}, Parke Davis, Solna, Sweden). The animals were fixed to the stereotaxic frame (David Kopf Instruments, Tujunga, U.S.A.). The skull was exposed and a permanent stainless cannula (22 gauge, length 18 mm) was implanted with its tip 1 mm above the third ventricle in the midline 6 mm below the bregma, according to the brain atlas of Luparello (1967). The guide cannula was fixed to the skull with screws and dental acrylic cement. The cannula was closed with a 18-mm-long stainless-steel stylet. After the surgical operation, animals were allowed to recover at least for 7 days. The animals were handled and weighed daily to habituate them to a partial restraint experience during i.c.v. infusions.

In vivo *studies*

At 1 h before the drug administration, animals were moved into clean cages and food jars were removed. Saline, pNPY (3.6 nmol), [Arg^6,Pro^{34}]pNPY (3.6 and 10 nmol), [Phe^7,-Pro^{34}]pNPY (3.6 and 10 nmol), [Ala^{31},Aib^{32}]pNPY (3.6 and 10 nmol) or [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP (0.9 and 3.6 nmol) were infused i.c.v. at a rate of 5 μl min^{-1} using a Hamilton infusion pump and syringes and an injection needle projecting 2 mm below the tip of the guide cannula. Infusion volume was 10 μl. After the drug administration, the infusion cannula was left in place for an additional 1 min to avoid back diffusion along the cannula. The animals were returned to their cages and food consumption was measured 1, 2, 3 and 4 h postinfusion. Food spillage was collected and subtracted from the intake. A video camera placed above the cage recorded the entire experiment. Afterwards, different eating parameters including latency to first meal (s), latency to drink (s), time

spent on eating (min), and number of meals and meal durations (s) were analysed from the video tapes. A meal was defined as an active eating episode from the first bite of food to the moment the animal left the food container. Maximum latency of 300 s was used for those animals not eating/drinking during the first 5 min of the experiment. Each animal received 3–5 different treatments with a 5–6 days recovery period between tests. At the end of the experiments, dye was infused i.c.v. and the staining of the third ventricle was examined.

Calculations and statistical analysis

The mean and standard error of the mean (s.e.m.) were calculated. The statistical differences between groups were determined with one-way analysis of variance followed by the *post hoc* comparisons with the test of Dunnet. When the presumptions of the one-way of analysis of variance were not fulfilled, the nonparametric Kruskal–Wallis test followed by the Mann–Whitney *U* test was used.

Results

Binding studies

Representative competition curves are shown in Figure 1. As expected, [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY bound with very high affinity to the guinea Y_1 receptor *in vitro*. The K_i values of [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY at the Y_1 receptor were 0.04 and 0.06 nM, respectively (Table 1). The K_i values of [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY at the Y_5 receptor were 1.38 and 4.65 nM, corresponding to 35- and 78-fold stronger binding to the Y_1 than to the Y_5 receptor (Table 1). Both compounds showed poor affinity for the Y_2 receptor (Table 1).

[Ala^{31},Aib^{32}]pNPY bound to the guinea pig Y_5 receptor with a K_i of 7.42 nM, whereas the K_i values of Y_1 and Y_2 receptors were 1700 and 63 nM, respectively (Table 1). The other compound, [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP, turned out to be as potent at the guinea pig Y_5 receptor as the native ligand NPY. It showed poor affinity for the Y_1 and Y_2 receptors (Table 1).

In vivo *study*

NPY at the dose of 3.6 nmol produced a statistically significant increase in the amount of food consumed (Figures 2, 3). In addition, it increased the time spent on eating and the number of meals (Figure 3). NPY treatment tended to decrease latency to eat, the average meal size and meal duration (Figure 4) compared to the controls, but caused no change in eating rate (Table 2). NPY administration doubled the water consumption but had no effect on latency to drink (Table 2).

Both of the Y_1-preferring compounds at the doses of 3.6 and 10 nmol stimulated food intake. The compounds increased the amount of food consumed, the time spent on eating and the number of meals (Figure 3). At the dose of 3.6 nmol, both Y_1-preferring compounds were equipotent with NPY (Figure 2). Both Y_1 agonists tended to decrease the average meal size and meal duration (Figure 4), and the latency to eat tended to be shorter in animals treated with the higher doses of theY_1 agonists (Figure 4). The compounds had no effect on eating rate or latency to drink, although they increased water intake (Table 2).

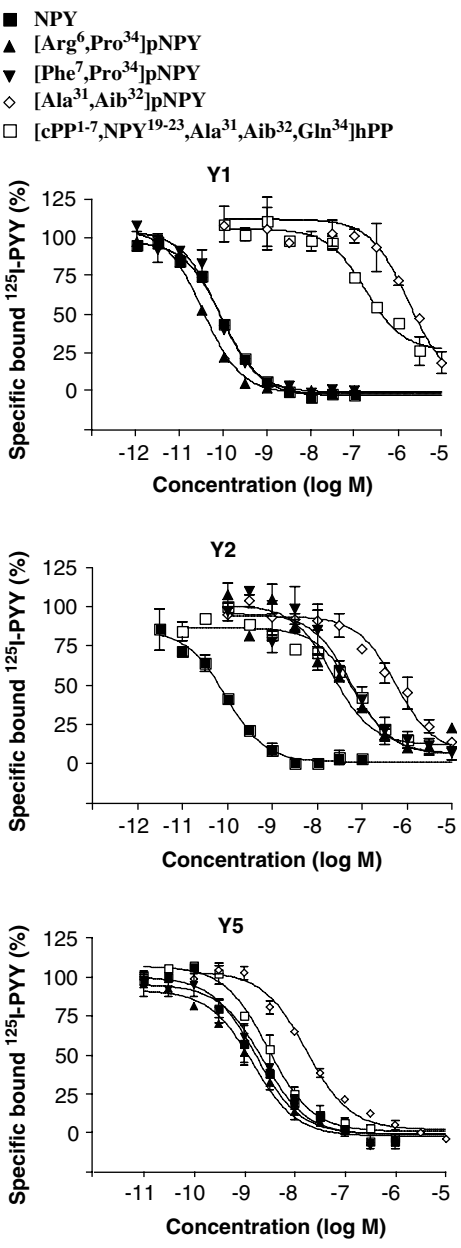

Figure 1 Competition of ^{125}I-pPYY binding by Y_1 and Y_5 agonists to membranes expressing the guinea pig Y_1, Y_2 and Y_5 receptors. Results shown are from one typical experiment performed in duplicate. Nonspecific binding was defined in the presence of 100 nM pNPY. K_i values are listed in Table 1.

The Y_5 receptor-preferring agent [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP at the dose 3.6 nmol produced approximately two-fold increase in food intake compared to NPY-induced intake (Figure 2). The compound also increased the eating time (Figure 3). Although the number of meals after [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP treatment was increased, the change was not statistically significant (Figure 3). The average meal duration as well as the meal size in guinea pigs treated with 3.6 nmol of [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32}, Gln^{34}]hPP was markedly higher than in groups treated with other agonists

Table 1 Binding affinities (Ki, nM) of the Y_1- and Y_5-receptor-preferring agonists for the guinea pig Y_1, Y_2, and Y_5 receptors

	Y_1	Y_2	Y_5	$K_i\ (Y_1) : K_i\ (Y_5)$
NPY	0.07±0.01	0.03±0.01	1.66±0.46	1:24
[Arg^6,Pro^{34}]pNPY	0.04±0.02	2.10±0.59	1.38±0.09	1:35
[Phe^7,Pro^{34}]pNPY	0.06±0.02	8.30±0.46	4.65±1.72	1:78
[Ala^{31},Aib^{32}]pNPY	1700±600	63±31	7.42±3.45	229:1
[cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP	85±55	6.10±3.70	1.32±0.61	64:1

Means + s.e.m. ($n = 3–6$).

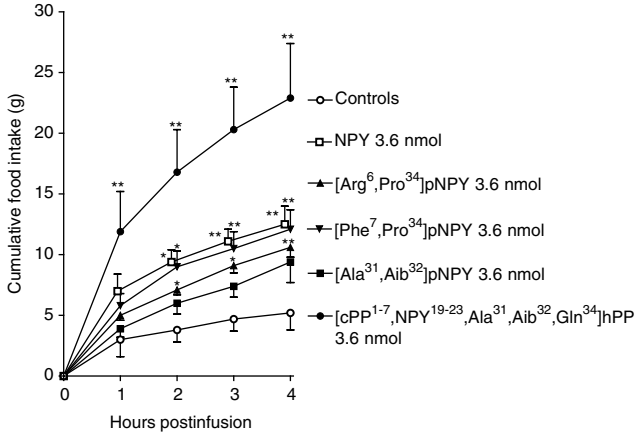

Figure 2 Cumulative food intake induced by 3.6 nmol of NPY, [Arg^6,Pro^{34}]pNPY, [Phe^7,Pro^{34}]pNPY, [Ala^{31},Aib^{32}]pNPY or [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP following i.c.v. administration to conscious guinea pigs. Mean±s.e.m., $n = 8–10$ in each group. Points marked with * are statistically different from the controls: *$P<0.05$; **$P<0.01$.

(Figure 3). [Ala^{31},Aib^{32}]pNPY, the other Y_5-preferring compound, had only a modest effect on food consumption and different feeding parameters (Figures 2–4). Neither of the Y_5-preferring peptides caused any marked changes in the latency to eat, but in [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP-treated guinea pigs, the latency to eat was significantly longer than in NPY-treated animals (Figure 4). [Ala^{31},Aib^{32}]pNPY at the dose of 10 nmol increased water consumption, while [cPP$^{1-7}$, NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP had no effect on water intake. Neither of the compounds had any effect on latency to drink (Table 2).

Discussion

In the present study, central administration of 3.6 nmol NPY to guinea pigs stimulated food intake. The result is in good accordance with our previous study (Lecklin *et al*., 2002) using a dose range from 0.9 up to 10.8 nmol of NPY. It has been reported that NPY decreases latency to initiate feeding in rats (Clark *et al*., 1987). Guinea pigs, unlike rats, do not show diurnal fluctuation in their feeding pattern and they eat as much during the light as the dark period (Hirch, 1973), and therefore the latency to eat can be short during the light phase when the animals are active. Although NPY treatment tended to decrease latency to eat, the changes were not statistically significant compared to the controls. NPY administration increased the number of meals in guinea pigs, a finding which

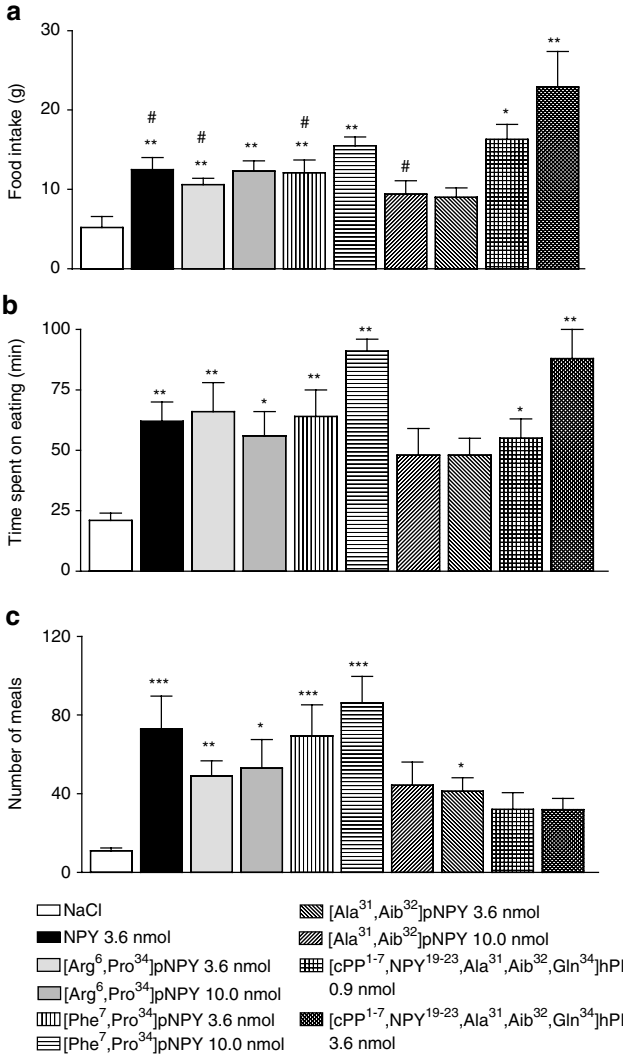

Figure 3 Food intake (upper panel), eating time (middle panel) and number of meals (lower panel) after i.c.v. administration of NPY, the Y_1-preferring [Arg^6,Pro^{34}]pNPY or [Phe^7,Pro^{34}]pNPY and the Y_5-preferring [Ala^{31},Aib^{32}]pNPY or [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31}, Aib^{32},Gln^{34}]hPP to conscious guinea pigs. Mean±s.e.m., $n = 8–10$ in each group. Columns marked with * are statistically different from the controls: *$P<0.05$; **$P<0.01$; ***$P<0.001$ and those marked with # differ ($P<0.05$) significantly from the group treated with 3.6 nmol [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP.

is in line with those made in rats (Ammar *et al*., 2000). A high number of meals in NPY-treated animals may reflect generalised behavioural activation, but it may as well reflect the reward produced by the initiation of a meal. It has been

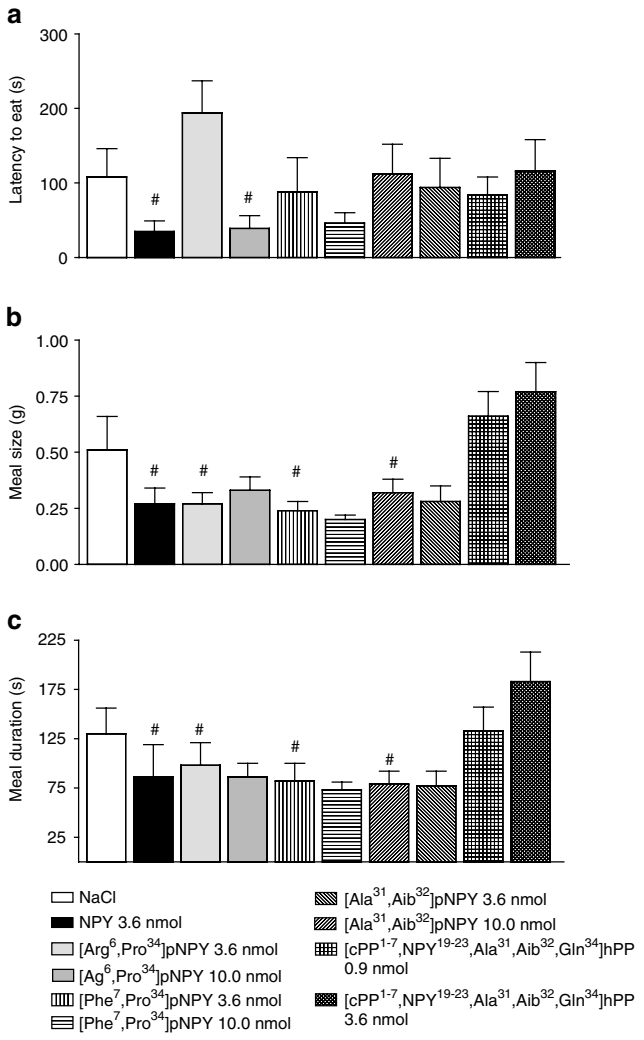

Figure 4 Latency to eat (upper panel), average meal size (middle panel) and meal duration (lower panel) after i.c.v. administration of NPY, the Y_1-preferring [Arg^6,Pro^{34}]pNPY or [Phe^7,Pro^{34}]pNPY and the Y_5-preferring [Ala^{31},Aib^{32}]pNPY or [cPP^{1-7},NPY^{19-23},Ala^{31},Aib^{32},Gln^{34}]hPP to conscious guinea pigs. Mean ± s.e.m., $n = 8-10$ in each group. Columns marked with # differ ($P < 0.05$) from the group treated with 3.6 nmol [cPP^{1-7},NPY^{19-23},Ala^{31},Aib^{32},Gln^{34}]hPP.

reported that NPY enhances the motivation to respond to rewarding stimuli such as eating (Brown *et al*., 2000). In the study of Flood & Morley (1991), NPY-treated rats that were allowed to choose between palatable food and regular chow, tolerated foot shocks or other aversive stimuli to get access to palatable food. In spite of the increased meal number, NPY tended to decrease the average meal size and duration and caused no change in the eating rate, three parameters that are connected to the consummatory phase. It seems therefore that exogenously applied NPY influences the appetitive phase rather than the consummatory phase of feeding in a similar way in guinea pigs as reported earlier for rats.

The Y_1-preferring peptides [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY displayed 35- and 78-fold higher affinity for the Y_1 over the Y_5 receptor, respectively, in cell membranes transfected with the guinea pig Y_1 or Y_5 receptor subtypes. The peptides behaved as full agonists when tested in human Y_1-receptors-expressing cells, and [Arg^6,Pro^{34}]pNPY was slightly less potent in inhibiting cAMP production than [Phe^7,Pro^{34}]pNPY and NPY, although they all bound with similar affinities for the Y_1 receptor (Söll *et al*., 2001). When [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY were infused i.c.v. to guinea pigs, they both stimulated food intake. When studied at equimolar doses, they were as effective as NPY. The finding is in agreement with previous studies using other Y_1-preferring agents, such as [D-Arg^{25}]-NPY and [D-His^{26}]-NPY, which were found to stimulate food intake in rats (Mullins *et al*., 2001). Since both [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY as well as NPY displayed nanomolar affinities to the Y_5 receptor subtype, we cannot completely rule out the possibility that the feeding elicitation was due to the activation of the Y_5 receptor. This, however, seems less likely, because numerous laboratories including ours have shown that selective Y_1 antagonism inhibits NPY-induced feeding (Wieland *et al*., 1998; Larsen *et al*., 1999; Duhault *et al*., 2000; Polidori *et al*., 2000; Kanatani *et al*., 2001; Lecklin *et al*., 2002). The Y_1 antagonism also inhibits [D-Arg^{25}]-NPY-induced feeding (Mullins *et al*., 2001).

In guinea pigs, [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY increased the number of small meals in a manner similar to NPY. They tended to decrease the latency to first meal but only when the high dose was used and they reduced the average meal size and duration. Similar feeding parameter profiles in NPY-treated and Y_1-agonist-treated guinea pigs indicate that Y_1 agonists, like NPY, stimulate the appetitive phase of feeding. The result also implies that NPY at the dose used in this study primarily elicits feeding through the Y_1 receptor. Studies of NPY-induced feeding in Y_1- and Y_5-receptor-deficient mice (Kanatani *et al*., 2001) also suggested that Y_1 is the major feeding receptor. Interestingly, the Y_1 knockout mice show reduced locomotor activity (Pedrazzini *et al*., 1998). Whether it is related to reduced food-seeking behaviour remains to be studied.

NPY and Y_1 agonists stimulated water intake. The latency to drink was not changed, which suggests that increased water intake could be a secondary response to increased food intake. Previously, it has been observed that NPY injected into the fourth ventricle stimulates water intake when food is present, but in absence of food it has no effect on drinking (Corp *et al*., 1990). Since Y_5 agonists did not stimulate water consumption, the changes in water intake seem to be related to the Y_1 receptor activation.

At present, several Y_5-preferring agents have been identified (Wyss *et al*., 1998; Cabrele *et al*., 2000; McCrea *et al*., 2000; Parker *et al*., 2000). In a recent study, the Y_5-preferring peptide 2–36[K^4,RYYSA$^{19-23}$]PP had a strong stimulatory effect on food intake (McCrea *et al*., 2000), while another agent, [D-Trp^{32}]NPY, caused only a modest response (Wyss *et al*., 1998). The Y_5-preferring agonists [cPP^{1-7},NPY^{19-23},Ala^{31},Aib^{32},Gln^{34}]hPP and [Ala^{31},Aib^{32}]pNPY that were used in this study have earlier been reported to elicit feeding in rats (Cabrele *et al*., 2000). In contrast to rat studies, [Ala^{31},Aib^{32}]pNPY had only a modest effect on food consumption in guinea pigs. This might be due to its slightly lower affinity than [cPP^{1-7},NPY^{19-23},Ala^{31},Aib^{32},Gln^{34}]hPP to the Y_5 receptor. Since the guinea pig Y_5 receptor displays 'only' 89% overall amino-acid identity to the rat receptor (Lundell *et al*., 2001), possibly the differences in the receptor structure might explain the unexpectedly weak responses in guinea pigs.

Table 2 Body weight, eating rate, latency to drink and water intake after i.c.v. infusion of NPY, Y_1-or Y_5-preferring agonists in conscious guinea pigs

	Body weight (g)	*Eating rate* ($g\,min^{-1}$)	*Latency to drink* (s)	*Water intake* ($ml\,4h^{-1}$)
Control	394±16	0.25±0.04	271±24	16.2±6.2
NPY 3.6 nmol	394±20	0.22±0.03	257±21	32.0±6.1**
Y_1-receptor-preferring agonists				
[Arg^6,Pro^{34}]pNPY 3.6 nmol	391±20	0.18±0.02	242±26	19.9±3.1
[Arg^6,Pro^{34}]pNPY 10.0 nmol	395±14	0.25±0.03	192±43	27.8±10.9*
[Phe^7,Pro^{34}]pNPY 3.6 nmol	390±19	0.21±0.02	242±37	39.7±10.2**
[Phe^7,Pro^{34}]pNPY 10.0 nmol	391±9	0.17±0.01	262±29	26.9±4.2*
Y_5-receptor-preferring agonists				
[Ala^{31},Aib^{32}]pNPY 3.6 nmol	395±20	0.22±0.02	247±28	22.0±6.0
[Ala^{31},Aib^{32}]pNPY 10.0 nmol	395±15	0.20±0.02	256±32	25.8±4.1*
[cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},G/n^{34}]hPP 0.9 nmol	392±20	0.30±0.02	213±59	16.8±3.1
[cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},G/n^{34}]hPP 3.6 nmol	388±26	0.25±0.02	247±35	20.0±4.1

Means±s.e.m., $n=8-10$. Values marked with * are significantly different from the controls: *$P<0.05$; **$P<0.01$.

The other Y_5-preferring agonist [cPP$^{1-7}$,NPY$^{19-23}$, Ala^{31},Aib^{32},Gln^{34}]hPP had a high affinity for the guinea pig Y_5 receptor. The signal transduction assays on human Y_5 receptors expressing cell line have shown that the compound acts as a full agonist and is at least as potent as NPY in inhibiting the forskolin-stimulated cAMP production (Cabrele *et al*., 2000). The result is in good correlation with the binding data. When examined *in vivo*, [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32}, Gln^{34}]hPP was generally twice as effective in stimulating food intake as any other of the compounds tested. It significantly increased the time spent on eating but had no effect on the number of meals. The average meal size and duration in animals treated with this Y_5 agonist (3.6 nmol) were markedly higher than those in animals treated with other compounds. [cPP$^{1-7}$, NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP had no effect on the latency to eat. It seems that this particular Y_5 agonist had a different action profile on feeding parameters compared to NPY or the Y_1 agonists. The peptide appears to influence the parameters associated with the consummatory phase of feeding. However, care should be exercised in the interpretation of the data, since NPY and its receptor agonists were infused i.c.v., and it is therefore possible that behavioural changes may differ if small doses of the compounds are injected directly into certain areas of the hypothalamus. As only one of the two Y_5 agonists tested had a clear orexigenic effect in guinea pigs, and as the present study was carried out with a rather limited number of animals, additional studies with other Y_5 agonists and possibly with other animal species are necessary.

Very recently it was reported that the blockade of the Y_5 receptor by a novel, nonpeptidergic Y_5 antagonist inhibited feeding induced by [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP but not that induced by NPY (Turnbull *et al*., 2002). Another new Y_5 antagonist also failed to block the effect of NPY on food intake in rats (Kanatani *et al*., 2000). In our previous study in guinea pigs (Lecklin *et al*., 2002), the Y_5 antagonist CGP 71683A attenuated NPY-induced feeding. The compound has also been shown to inhibit spontaneous food intake in diabetic, 24 h fasted and free-feeding rats and mice (Criscione *et al*., 1998; Kask *et al*., 2001). Nowadays, CGP 71683A has been questioned as an *in vivo* tool because of its activity at serotonin (5-HT) reuptake recognition site and cholinergic muscarinic receptors (Della Zuana *et al*., 2001) and its efficacy in NPY knockout mice (Bannon *et al*., 2000), and for the time being it is difficult to evaluate the importance of those results obtained using this compound. However, the present findings with [cPP$^{1-7}$,NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP clearly show that an activation of the receptor subtype Y_5 leads to increased food intake. The feeding response after [cPP$^{1-7}$, NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP seemed to be different from those seen after NPY or Y_1-preferring agonists, indicating that both receptor subtypes are involved in the stimulation of food intake, but through different mechanisms. Since NPY displays higher affinity for the Y_1 subtype than for the Y_5, one could expect that small amounts of NPY released would primarily activate Y_1 receptors in the brain areas where both receptor subtypes are present. If activation of Y_1 receptors alone is enough to induce feeding, as the prevailing data seem to suggest, the initiation of eating *via* feedback mechanisms would reduce the release of NPY back to the control level. According to this model, the Y_5 subtype would be activated (together with the Y_1 receptor) when NPY is released in high amounts or when exogenous NPY is administered in high doses. It has been shown that food deprivation and food restriction increase the release rate of NPY (Kalra *et al*., 1991b) and some studies (Schaffhauser *et al*., 1997; Widdowson *et al*., 1997) have related the Y_5 receptor activation to fasting- and food restriction-induced hyperphagia. Fasted animals most probably would eat large and long-lasting meals similar to those seen after the administration of [cPP$^{1-7}$, NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP. Unfortunately, the data from Y_1 and Y_5 knockout mice do not support this hypothesis since fasting-induced food intake was reduced in Y_1, but not in Y_5, knockout mice (Marsh *et al*., 1998; Pedrazzini *et al*., 1998). Alternatively, the Y_1 and Y_5 receptors may be activated under different conditions as it has been suggested earlier (Balasubramaniam *et al*., 2002). Thus, further studies on the effects of NPY and its receptor ligands on various aspects of feeding are needed to better understand the roles that Y_1 and Y_5 receptor subtypes play in a complex system regulating food intake.

In conclusion, two novel Y_1-receptor-preferring peptides, [Arg^6,Pro^{34}]pNPY and [Phe^7,Pro^{34}]pNPY, were tested *in vivo* for the first time and were found to stimulate feeding in guinea pigs. Furthermore, the Y_5-preferring agonist [cPP$^{1-7}$, NPY$^{19-23}$,Ala^{31},Aib^{32},Gln^{34}]hPP very potently increased food intake. The present data provide further evidence for the hypothesis that the receptor subtypes Y_1 and Y_5 are both involved in the stimulation of food intake. The Y_1 and Y_5 agonists had distinct action profiles on various feeding

parameters, suggesting that they might modify different phases of feeding behaviour.

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