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Evidence for involvement of neuropeptide Y receptors in the regulation of food intake: studies with Y_1-selective antagonist BIBP3226

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1 Experiments were conducted to evaluate the effects of the novel non-peptide neuropeptide Y Y_1 receptor antagonist, BIBP3226 (N^2-(diphenylacetyl)-N-[(4-hydroxy-phenyl)methyl]-D-arginine amide) on spontaneous, fasting-induced and NPY-induced food intake in rats. In addition to consumption of regular chow, the effects of BIBP3226 on consumption of highly palatable sweetened mash were monitored in a 1 h test on first exposure and after familiarization with novel food.

2 BIBP3226 (10.0 nmol, i.c.v.) had no effect on the consumption of regular chow, but reduced significantly the intake of highly palatable diet and the food intake stimulated by fasting (24 h). Neuropeptide Y (NPY, 1.0 nmol, i.c.v.) significantly increased the consumption of regular rat chow. This orexigenic effect of NPY was blocked by BIBP3226 (10.0 nmol, administered i.c.v. 5 min before NPY) at 30 min and 4 h, but not at 1 and 2 h. When animals were pretreated with diazepam (0.5 mg kg^{-1}, i.p., 20 min before NPY), BIBP3226 failed to suppress NPY-induced feeding.

3 An NPY Y_1 and Y_3 receptor agonist, [Leu^{31},Pro^{34}]NPY and a Y_5 receptor agonist human peptide YY_{3-36} ($hPYY_{3-36}$, both 30 pmol), microinjected into the paraventricular nucleus of the hypothalamus (PVN) increased the consumption of regular rat chow. BIBP3226 (0.4 nmol, into the PVN) completely blocked the stimulatory effect of [Leu^{31},Pro^{34}]NPY but not that of $hPYY_{3-36}$. BIBP3226 (0.4 nmol) alone failed to modify the consumption of the regular chow. Higher doses of BIBP3226 (1.0 and 2.0 nmol) injected into the vicinity of the PVN reduced the consumption of the sweetened mash.

4 These results suggest that both the NPY Y_1 and Y_5 receptors in the PVN are involved in the regulation of food intake. The stimulatory effect of exogenous NPY is probably mediated through an NPY receptor subtype that is not identical with the Y_1 receptor (possibly Y_5 receptor). However, the NPY Y_1 receptors may mediate the effect of endogenous NPY in conditions of increased energy demand or on intake of highly palatable diets.

Keywords: Neuropeptide Y (NPY); Y_1 receptor; Y_5 receptor; [Leu^{31},Pro^{34}]NPY; PYY_{3-36}; neuropeptide Y Y_1 antagonist; BIBP3226; diazepam; food intake; anxiety; paraventricular nucleus of hypothalamus

Introduction

Neuropeptide Y (NPY), a 36-amino acid peptide, is widely distributed in the central nervous system (Chronwall *et al*., 1985) and intraventricular or intra-hypothalamic administration of NPY has been shown to stimulate food intake (Stanley, 1993). The sequences of rat Y_1, Y_2, Y_4 and Y_5 receptor cDNAs have been identified (Eva *et al*., 1990; Krause *et al*., 1992; Gerald *et al*., 1996; Lundell *et al*., 1996; St-Pierre *et al*., 1998), whereas putative Y_3 receptors (Grundemar *et al*., 1991) at which NPY is at least 10 times more potent than peptide YY (PYY) have not been cloned yet. NPY, pancreatic polypeptide (PP), PYY and their truncated amino-acid substituted analogues differ in potency at NPY receptor subtypes (Gerald *et al*., 1996; Michel *et al*., 1998). These peptides have been used to identify the NPY receptor subtype involved in feeding (Kalra *et al*., 1991; O'Shea *et al*., 1997). The NPY Y_1, Y_2 and Y_5 receptors are found in hypothalamic areas where NPY administration evokes a feeding response in rats (Gerald *et al*., 1996; Hu *et al*., 1996; Gehlert & Gackenheimer, 1997). The robust effects of NPY on food intake are believed to be mediated through the receptor subtype that is similar, but not identical to the Y_1 receptor. Thus, both the full molecule of NPY and the Y_1 agonist [Leu^{31},Pro^{34}]NPY increase food intake whereas the Y_2 agonist NPY_{13-36} is inactive (O'Shea *et al*., 1996; Kalra *et al*., 1991). Human PYY and PP also stimulate food intake although they bind to the NPY Y_1 receptor with much lower affinity than intact NPY (Gerald *et al*., 1996). The pharmacological *in vitro* profile of the recently cloned rat NPY Y_5 receptor is distinct from other NPY subtypes and the affinities of various agonists closely correlate with their *in vivo* potency after i.c.v. administration in feeding assays suggesting that the NPY Y_5 receptor may be the long-sought after 'feeding' receptor (Gerald *et al*., 1996).

Due to the lack of specific antagonists it has been difficult to establish which NPY receptor(s) subtype(s) is/are involved in the regulation of food intake. Putative Y_1 antagonists, PYX-2 (Leibowitz *et al*., 1992) and [D-Trp^{32}]NPY (Balasubramaniam *et al*., 1994) have been shown to reduce spontaneous and NPY-stimulated food intake. Recent data, however, indicate that both of these compounds are weak antagonists at NPY Y_1 receptors (Palea *et al*., 1995; Wieland *et al*., 1995) and high doses of [D-Trp^{32}]NPY may behave as agonists *in vivo* (Gerald *et al*., 1996; Matos *et al*., 1996). The inhibition of NPY synthesis or NPY Y_1 receptor synthesis by antisense oligonucleotides has yielded controversial results. NPY antisense injected i.c.v. or into the arcuate nucleus reduces food intake (Akabayashi *et al*., 1994; Hulsey *et al*., 1995) although an increase in food intake has also been reported after i.c.v.

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administration of NPY antisense (Lopez-Valpuesta *et al*., 1996). Furthermore, antisense oligonucleotides targeted against the NPY Y_1 receptor increase food intake (Heilig, 1995; Lopez-Valpuesta *et al*., 1996).

The aim of the present study was to evaluate the effects of the highly selective non-peptide Y_1 selective antagonist BIBP3226 (Rudolf *et al*., 1994; Doods *et al*., 1996) on food intake. BIBP3226 failed to affect forskolin-stimulated cyclic AMP production by NPY in cells transfected with Y_2, Y_4 or Y_5 receptors (Gerald *et al*., 1996) confirming the selectivity of the compound. In the same study BIBP3226 had no effect on NPY-induced feeding. However, recently BIBP3226 has been shown to antagonize NPY-induced feeding in rats (O'Shea *et al*., 1997). The role of the NPY Y_1 receptor in feeding behaviour is therefore unclear and these contradictory findings prompted us to study the effects of BIBP3226 on food intake.

We have shown that BIBP3226 has anxiogenic-like effects in the elevated plus-maze test (Kask *et al*., 1996; 1997). In the present investigation we studied whether an anxiogenic dose of BIBP3226 affects spontaneous food intake, fasting-induced and NPY-induced feeding. In order to test the possibility that the antagonism of NPY-induced feeding by BIBP3226 (O'Shea *et al*., 1997) may be due to the anxiogenic effects of BIBP3226 (Kask *et al*., 1996; 1997) rats were pretreated with diazepam. It has been suggested that activation of the Y_1 receptor mediates the preference for carbohydrate rich diets (Leibowitz & Alexander, 1991). Therefore, the effect of BIBP3226 on consumption of highly palatable food has been studied. Since in preliminary experiments BIBP3226 blocked NPY-induced feeding (see also O'Shea *et al*., 1997), the possibility that both the Y_5 and Y_1 receptors should be activated for the feeding response was addressed by measuring the effects of BIBP3226 on feeding induced by peri-PVN injection of two agonists of the NPY receptors, [Leu^{31},Pro^{34}]NPY and human PYY_{3-36} ($hPYY_{3-36}$). These NPY agonists have different selectivity towards the NPY Y_1 and Y_5 receptors (Gerald *et al*., 1996) and are now recommended for characterization of the effects mediated by the NPY receptor activation (Michel *et al*., 1998).

A preliminary report of these data has been presented at the 4th International NPY Conference, October 13–14, 1997, London, U.K.

Methods

Animals and diets

Male Wistar rats (Grindex, Latvia) weighing 240–400 g at the time of surgery were individually housed in hanging wire-mesh cages ($45 \times 37 \times 19$ cm) under controlled light (the lights on from 08:00 h to 20:00 h) and temperature (20–22°C), with free access to tap water and food (Lactamin, R35, Sweden).

Surgery and animal care

The rats were anaesthetized by i.p. injection of chloral hydrate solution (350 mg 10 ml^{-1} kg^{-1}) and fixed in a sterotaxic frame (Kopf model 900, David Kopf Instruments, Tujunga, CA, U.S.A.). The skull was exposed and 2% lidocaine solution applied to the periost. Permanent stainless steel 25 gauge cannulae terminating 1 mm dorsally to the planned injection site were implanted. The correct placement of a cannula for i.c.v. injections was verified by gravity infusion during some implantations. The coordinates taken from the atlas of Paxinos & Watson (1986) for the PVN were A: −0.4, L: −0.4; V: −7.2, incisor bar +3.0 and for the lateral cerebral ventricle A: +0.7, L: +1.4, V: −3.2–3.8, with incisor bar at +3.5. The 25 gauge guide cannula was anchored to the skull with three stainless steel screws and dental acrylic cement, and closed with a stylet when not in use. Rats were allowed to recover for 7 days and were handled and weighed daily to habituate them to the partial restraint experienced during intracerebral injection. Rats losing more than 10% of their body weight during the postoperative period and not regaining it during 1 week were not included in the study ($n = 8$).

Procedure and injections

On test days the procedure was identical in all experiments. The food was removed 1 h or 24 h (food deprivation experiment) before i.c.v. or intra-PVN injections. Each rat was then removed from the cage and injected. Drugs or vehicle were injected over 2 min, through 33 gauge injector connected to the 10 μl SGE syringe and infusion pump (World Precision Instruments, Sarasota, U.S.A.) with PE10 or PE20 tubing. The movement of an air bubble in the tubing confirmed the drug flow. Following the injection, the needle was left in place for an additional 30 s and the cannula was closed with a stylet. Immediately after the injection, the rats were returned to the home cage. A preweighed amount of food pellets (approximately 20 g) or sweetened mash (approximately 30 g) was made available on clean Petri dishes after the last injection. The remaining food and any spillage, collected from stainless steel plates placed beneath the cages, was measured to the nearest 0.01 g using a Mettler balance. Food weights were corrected for spillage at each measurement. All treatments were given at least 48 h apart, between 1600 h and 2000 h.

Experimental protocols

Effects of i.c.v. administration of BIBP3226 on food intake in free-feeding rats Rats (280–330 g) were injected with saline or BIBP3226 (10 nmol in 6.5 μl), returned to home cages and and given pre-weighed food pellets on clean Petri dishes. The food intake was measured after 0.5, 1, 2, 4 and 12 h.

Effects of i.c.v. administration of BIBP3226 on food intake in food-deprived rats Rats (240–310 g) were deprived of food for 24 h. On the next day the rats were injected i.c.v. with BIBP3226 (1 and 10 nmol in 6.5 μl) or saline and returned to home cages with pre-weighed food pellets. Remaining food and spillage were measured at 0.5, 1, 2, 4 and 12 h.

Effects of single and combined administration of NPY and BIBP3226 on food intake in free-feeding rats In a separate group of non-deprived rats (280–310 g) feeding was induced with NPY (0.2 and 1 nmol in 5 μl). To investigate the potential antagonistic activity of BIBP3226 on the NPY-induced increase in feeding behaviour, BIBP3226 (10 nmol in 6.5 μl) or saline was injected 5 min before NPY injection (1 nmol, i.c.v.). This interval was selected because rats start to eat within 10–15 min after i.c.v. injection of 0.2 and 1 nmol of NPY (Stricker-Krongrad *et al*., 1996) and the half-life of BIBP3226 is short (Malmström *et al*., 1997). Immediately after the second injection, the rats were placed into the home cages, and the amount of food consumed by each animal was measured at 0.5, 1, 2, 4 and 12 h.

Effects of diazepam on the BIBP3226-induced suppression of NPY-induced feeding To address the question whether

BIBP3226 reduces food intake by indirect/physiological antagonism (by generating anxiety), the rats (280–330 g) were pretreated with diazepam (0.5 mg kg^{-1} i.p. in a volume of 2 ml kg^{-1}) or vehicle 20 min before the food presentation. Five minutes before the test the rats were given an infusion of BIBP3226 (10 nmol, i.c.v.) or saline into the lateral ventricle and finally NPY (1 nmol) or saline was infused through the same cannula. Thus, the rats received one of the following combinations: (1) vehicle + saline + saline; (2) vehicle + BIBP3226 + saline; (3) vehicle + saline + NPY; (4) vehicle + BIBP3226 + NPY; (5) diazepam + BIBP3226 + NPY; (6) diazepam + saline + saline. The combination diazepam + saline + NPY was not used. It should be noted however, that even when a higher dose of NPY (2 nmol) was used, a similar profile of feeding response was observed with a comparable diet (Paez *et al*., 1991) thus potentiation of the effect of NPY by diazepam should not be expected. Remaining food and spillage was measured at 0.5, 1 and 2 h.

Effects of i.c.v. and intra-PVN administration of BIBP3226 on consumption of highly palatable sweet mash Non-deprived rats (350–400 g) were tested for consumption of sweetened mash in 1 h tests. The test meal was made up daily according to the following formula: 400 g of milled regular rat chow (R70, Lactamin, Sweden), 250 ml of distilled water and 50 ml of sweetened chocolate flavoured condensed milk. The constituents were mixed to produce a soft mash 1 h before the experiment. One group of rats ($n=11$) was injected with saline or BIBP3226 (10 nmol in 6.5 μl, i.c.v.). Another group of rats ($n=10$) was familiarized over a period of 4 days to eating a sweetened mash in daily 1 h tests preceded by i.c.v. injections of saline, by which time the level of food intake had reached a stable level. Starting from the fifth day the rats were injected with BIBP3226 (0.1, 1.0 and 10.0 nmol in 6.5 μl, i.c.v.). Rats with cannulae aimed at the PVN ($n=10$) received injections of saline or BIBP3226 (0.5, 1.0 nmol in 0.65 μl and 2.0 nmol in 1.3 μl). After injection rats were returned to home cages and the amount of remaining mash and spillage was measured. Regular pellets remained available throughout the testing period. A minimum of 2 days elapsed between successive drug treatments and the treatments were administered in a randomized order.

Effects of [Leu^{31},Pro^{34}]NPY, $hPYY_{3-36}$ and BIBP3226 microinjected into paraventricular nucleus on spontaneous food intake The rats (350–400 g, $n=15$) with cannulae aimed at the PVN received the following drug combinations: (1) saline; (2) [Leu^{31},Pro^{34}]NPY or $hPYY_{3-36}$ (both 30 pmol); (3) BIBP3226 (0.4 nmol); (4) mixture of [Leu^{31},Pro^{34}]NPY or $hPYY_{3-36}$ and BIBP3226 (30 pmol and 0.4 nmol, respectively). Injections were given in a volume of 0.65 μl. The food intake was measured in a 1 h test. A minimum of 2 days elapsed between successive drug treatments.

Verification of injection sites

At the end of the study, the rats were overdosed with chloral hydrate and methylene blue dye was injected to mark the injection site. The brains were removed and fixed in 10% buffered neutral formalin or inspected for distribution of the dye. Fixed tissues were embedded in paraffin and sliced at 15 μm, mounted and stained with hematoxilin-eosin. Histological sections were examined microscopically. The rats with injection sites within or close to the PVN or with uniform distribution of the dye in the ventricles were used for data analysis. Two animals in which the dye was not injected due to clogging of the cannulae, but which responded earlier to i.c.v. injection of NPY with vigorous feeding were also included. The percentage of correctly placed i.c.v. cannulae varied from 70% in first experiments to 95% when cannulae were implanted under gravity infusion. All PVN cannulae were accurately located (Figure 1). In addition to histological verification of injection sites, 10 satiated rats with PVN cannulae were injected with $hPYY_{3-36}$ before dye injection. Food intake was increased in all rats above 2.5 g in a 1 h test, suggesting that the injection sites remained responsive to $hPYY_{3-36}$ over time.

Side effects

The main drawback of BIBP3226 is that after i.c.v. or PVN application unwanted side effects appear (Doods *et al*., 1996). In some cases (<5% of correctly localized injections) turning behaviour (circling and barrel-rolling) was observed after i.c.v. injection of BIBP3226 (10 nmol only). One BIBP3226-treated rat, instead of eating, started to 'play' with the Petri dish during the palatable food test. These rats were excluded from data analysis. We did not observe any toxic effects after microinjections of BIBP3226 into the PVN.

Drugs

BIBP3226, (N^2-(diphenylacetyl)-N-[4-hydroxy-phenyl)methyl]-D-arginine amide) was dissolved in saline (0.77 mg ml^{-1}) after brief sonication. NPY, [Leu^{31},Pro^{34}]NPY and $hPYY_{3-36}$, all from Bachem (Bubendorf, Switzerland), were dissolved in distilled water to provide a concentration of 100 pmol μl^{-1} and stored in aliquots at $-20^{\circ}C$, final dilutions were made with saline. Diazepam (Hoffmann-LaRoche, Switzerland) was dissolved in distilled water to which a drop of Tween-80 had been added. The doses of i.c.v. BIBP3226 and i.p. diazepam were selected on the basis of earlier studies (Gerald *et al*., 1996; Kask *et al*., 1996). The dose of intra-PVN [Leu^{31},Pro^{34}]NPY was selected on the basis of the study by Kalra *et al*. (1991) as a minimal effective dose producing a reliable feeding response. The doses of intra-PVN BIBP3226 were maximal possible and were limited by the poor solubility of BIBP3226 and limitations in the injection volume.

Statistical evaluation

The data from food intake tests are expressed as cumulative values and are represented as mean ± s.e.mean. Data were analysed by one-way analysis of variance (ANOVA) followed by Scheffe's test where appropriate with the exception of two experiments (spontaneous food intake and first exposure to sweet mash) where two groups were compared using Student's *t*-test. Differences were considered to be significant when $P<0.05$.

Results

Effects of the intraventricular administration of BIBP3226 on food intake in free-feeding rats

The potentially anxiogenic dose of BIBP3226 (10 nmol), administered i.c.v. immediately before the test, did not significantly alter food consumption over 4 h at any point of measurement. Food intake during the first 30 min of the test was 1.99 ± 0.57 g for saline treated ($n=7$) and 1.51 ± 0.36 g for

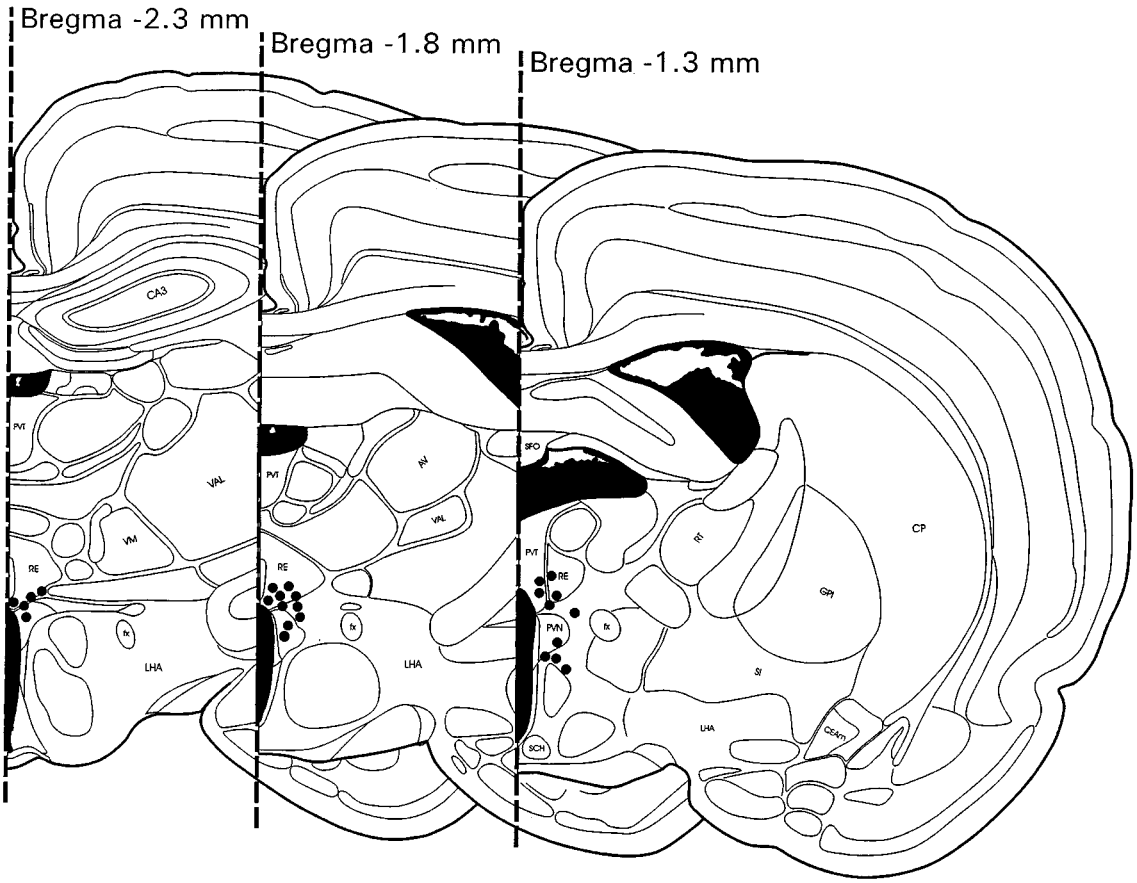

Figure 1 Diagram of coronal sections of rat forebrain: circles represent sites microinjected with [Leu^{31},Pro^{34}]NPY, $hPYY_{3-36}$ (both 30 pmol), BIBP3226 (0.5–2.0 nmol) or mixture of BIBP3226 (0.4 nmol) and NPY peptides (30 pmol). Plates are adapted from Swanson's atlas of rat brain (1992).

BIBP3226 (10.0 nmol) treated rats ($n=10$). This difference was not statistically significant.

Effects of the intraventricular administration of BIBP3226 on food intake in food-deprived rats

The effects of BIBP3226 (1.0 and 10.0 nmol, i.c.v. immediately before the test) on food intake over 12 h in food deprived (24 h) rats is shown in Figure 2. BIBP3226 had a significant effect on cumulative food intake over 4 h, [$F(2,28)=4.23$, $P<0.05$]. Individual comparisons with Scheffe's test revealed that the higher dose of BIBP3226 (10.0 nmol) significantly reduced the increase in food intake induced by 24 h food deprivation during the first 4 h of testing, while 1.0 nmol of BIBP3226 was without effect. The feeding occurring between 4 and 12 h after injection (feeding during the dark phase) was used as an additional measure to evaluate the effects of BIBP3226 on nocturnal feeding. By the 12th hour the total amount of food consumed during the dark-phase was not different between the groups.

Effect of single and combined administration of NPY and BIBP3226 on NPY-induced feeding in free-feeding rats

NPY (0.2 and 1 nmol) injected into the lateral ventricles produced a significant stimulation of feeding over the course of the following 4 h period [$F(2,10)=8{,}23$, $P<0.01$]. The increase in food intake in NPY-treated rats was evident at 30 min and the cumulative food intake was significantly higher in NPY-treated animals during the first 2 h. At 4 h the cumulative food intake was significantly increased only in rats treated with 1 nmol NPY. The effect of BIBP3226 (10.0 nmol) and NPY (1.0 nmol) alone and the combined administration of 10.0 nmol BIBP3226 (5 min before NPY) and 1.0 nmol NPY on cumulative food intake is shown in Figure 3. Repeated measures ANOVA revealed that these treatments had a significant effect on cumulative food intake over 4 h ($F3,34=21.99$ $P<0.005$). *Post-hoc* analysis indicated that food intake was increased both in NPY-treated rats and in rats pretreated with BIBP3226 before injection of NPY. Factorial ANOVA demonstrated that food intake was different between treatment groups at 0.5, 1, 2 and 4 h but not at 12 h. Individual comparisons with Scheffe's test revealed that i.c.v. administration of NPY induced a significant increase in food intake at 0.5, 1, 2 and 4 h. NPY-induced feeding was prevented by i.c.v. BIBP3226 pretreatment at 30 min, but not at 1 and 2 h. BIBP3226 decreased the effect of NPY also at 4 h. I.c.v. administration of BIBP3226 followed by saline injection did not affect food intake over the 12 h time period at any time point.

Effect of diazepam on the BIBP3226-induced suppression of NPY-induced feeding

The results of this experiment are shown in Figure 4. BIBP3226 (10.0 nmol, i.c.v.) alone did not affect food intake while NPY (1.0 nmol) significantly increased it. When the rats

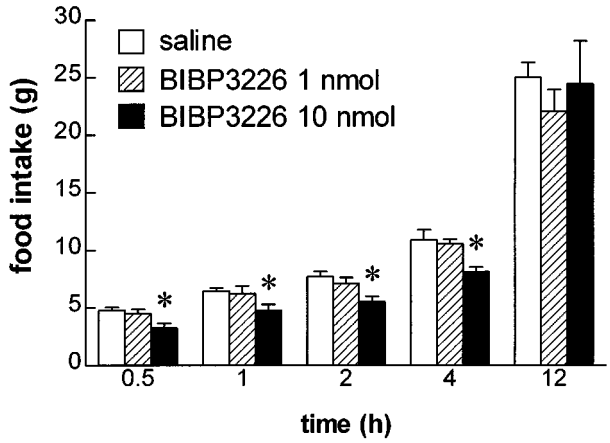

Figure 2 Cumulative food intake over 12 h in food deprived rats (24 h) after i.c.v. injection of saline ($n=16$) or BIBP3226 (1.0 nmol ($n=7$); 10.0 nmol ($n=8$). The drugs were infused in a volume of 6.5 μl immediately before the test. Columns represent mean cumulative food intakes (g) and bars show s.e.mean. *$P<0.05$ – significantly different from saline treatment (Scheffe's test).

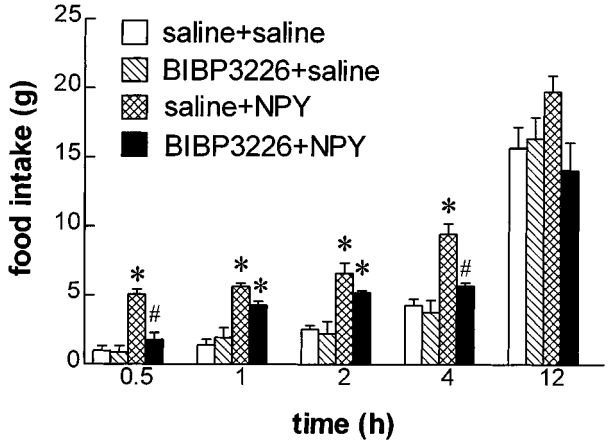

Figure 3 The effect of BIBP3226 on NPY-induced feeding over 12 h. BIBP3226 (10.0 nmol, i.c.v.) or saline was administered 5 min before NPY (1.0 nmol, i.c.v.). Thus, the rats were injected with following combinations of drugs: (1) saline + saline ($n=8$); (2) BIBP3226 + saline ($n=4$); (3) saline + NPY ($n=5$); (4) BIBP3226 + NPY ($n=4$). Results are shown as cumulative food intake in grams (mean ± s.e.mean). *$P<0.005$ – significantly different from controls. #$P<0.005$ – significantly different from rats treated with saline and NPY (Scheffe's test).

were treated with BIBP3226 5 min before NPY infusion, NPY failed to increase food intake during the first 30 min of the test replicating the data from the previous experiment. Subsequently, the food intake increased and at 1 and 2 h the food intake in rats treated with a combination of BIBP3226 and NPY was significantly increased compared to controls (Figure 4). The inhibitory effect of BIBP3226 on NPY-induced feeding, which was only evident at the 30 min time point, was completely abolished by diazepam pretreatment. Diazepam (0.5 mg kg^{-1}) itself tended to increase the food intake. The food intake in rats treated with diazepam was statistically different from both controls and NPY-treated rats at 2 h indicating that diazepam increased food intake at this point of measurement but this effect was considerably lower than the effect of NPY.

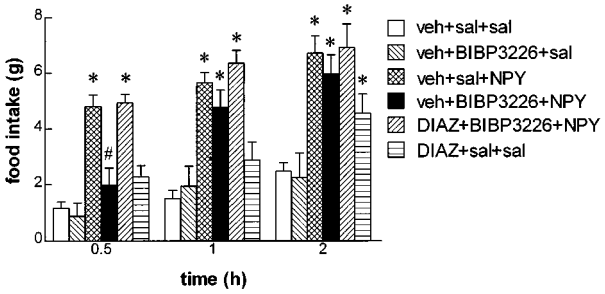

Figure 4 The effect of diazepam on BIBP3226-induced suppression of NPY-induced feeding. Diazepam (0.5 mg kg^{-1}) was injected i.p. 20 min before and BIBP3226 (10 nmol, i.c.v.) 5 min before NPY (1 nmol, i.c.v.) administration. Thus, the rats received one of the following combinations: (1) vehicle + saline + saline ($n=12$); (2) vehicle + BIBP3226 + saline ($n=5$); (3) vehicle + saline + NPY ($n=5$); (4) vehicle + BIBP3226 + NPY ($n=8$); (5) diazepam + BIBP3226 + NPY ($n=4$); (6) diazepam + saline + saline ($n=5$). After last injection rats were returned to home cages with preweighed food pellets. Results are presented as cumulative food intake (mean ± s.e.mean). *$P<0.005$ – significantly different from controls. #$P<0.005$ – significantly different from rats treated with vehicle + saline + NPY

Effect of i.c.v. and PVN administration of BIBP3226 on ingestion of sweet mash

Non-deprived rats were tested for consumption of sweetened mash in a 1 h test. Sweet mash was readily consumed on the first exposure but a stable intake was only observed by the 4th day. A potentially anxiogenic dose of BIBP3226 (10.0 nmol, i.c.v.) suppressed the consumption of sweet mash on the first exposure (7.29 ± 0.72 g and 3.1 ± 0.81 g for saline ($n=5$) and BIBP3226-treated ($n=5$) rats, respectively, $P<0.01$). The effects of BIBP3226 (0.1, 1.0 and 10.0 nmol/6.5 μl, i.c.v.) on consumption of sweet mash after habituation are shown in Figure 5a. BIBP3226 had a significant effect on sweet mash consumption ($F3,24=4.66$, $P<0.05$). *Post-hoc* analysis revealed that only the high dose of BIBP3226 (10.0 nmol) decreased food intake ($P<0.05$). A lower dose (1.0 nmol) tended to decrease mash consumption but this effect was not significant ($P=0.104$). The consumption of the sweet mash was also affected when BIBP3226 was injected into the vicinity of the PVN ($F3,26=5.99$, $P<0.005$). BIBP3226 (1.0 and 2.0 nmol, PVN) significantly reduced the consumption of the sweetened mash (Figure 5b).

The effect of [Leu^{31},Pro^{34}]NPY, hPYY$_{3-36}$ and BIBP3226 microinjected into paraventricular nucleus on spontaneous food intake

The effect of [Leu^{31},Pro^{34}]NPY (30 pmol), BIBP3226 (0.4 nmol), [Leu^{31},Pro^{34}]NPY-BIBP3226 mixture (30 pmol and 0.4 nmol, respectively) and vehicle are shown in Figure 6a. These treatments had a significant effect on food intake during a 1 h test period ($F3,25=12.19$ $P<0.005$). *Post-hoc* analysis revealed that [Leu^{31},Pro^{34}]NPY increased food intake ($P<0.01$), and this effect was completely abolished by coadministration of BIBP3226. An hPYY$_{3-36}$ and an hPYY$_{3-36}$-BIBP3226-mixture injected into the PVN had a significant effect on food intake in a 1 h test ($F3,34=6.88$ $P<0.001$) as shown in Figure 6b. *Post-hoc* analysis revealed that both treatments significantly increased food intake ($P<0.05$), thus BIBP3226 failed to affect the feeding response induced by hPYY$_{3-36}$. In both experiments BIBP3226 alone had no effect on food intake.

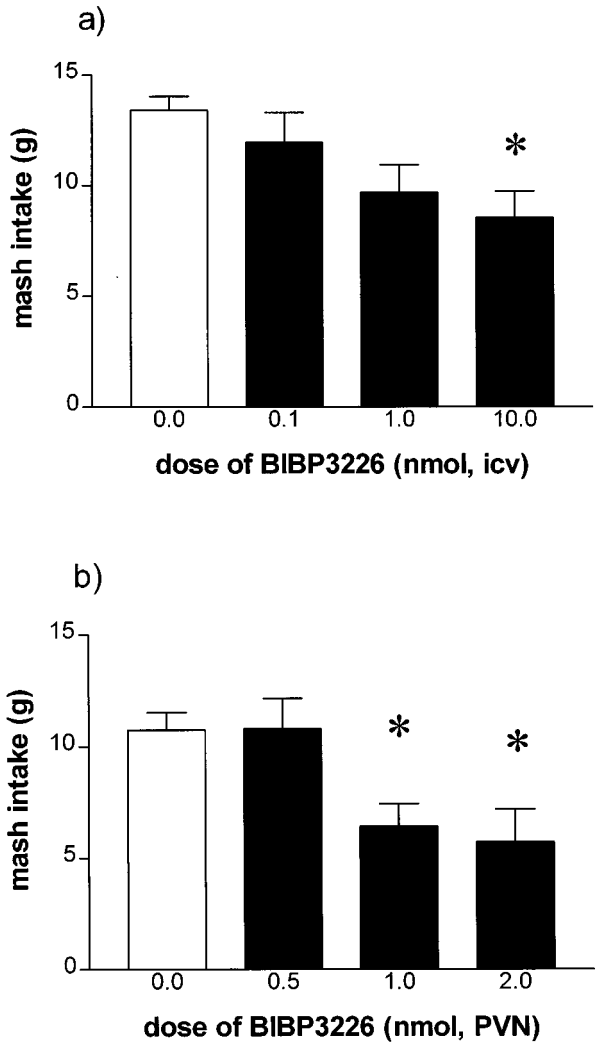

Figure 5 The effect of BIBP3226 on consumption of palatable sweet mash in habituated rats. BIBP3226 was injected (a) i.c.v. (0.1 ($n=5$), 1.0 ($n=7$) and 10.0 ($n=7$) nmol in 6.5 μl), or (b) into the vicinity of the paraventricular nucleus (0.5 ($n=6$), 1.0 ($n=8$) and 2.0 ($n=6$) nmol in 0.65 or 1.3 μl) immediately before the test. The results are shown in terms of mean intake of palatable food (g) in a 1 h test (s.e.mean shown by bars). $*P<0.05$-vs saline treated rats ($n=9-10$, Scheffe's test).

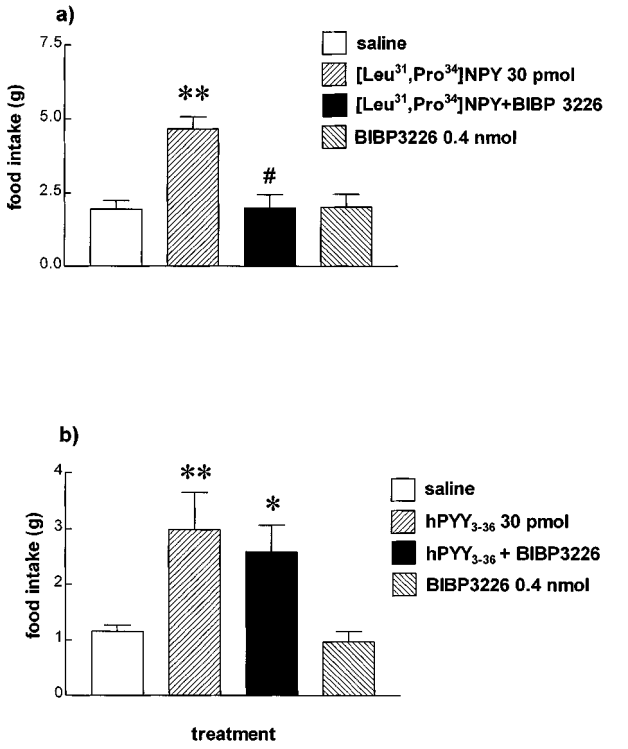

Figure 6 The effect of BIBP3226 on food intake induced by microinjection of (a) [Leu^{31},Pro^{34}]NPY ($n=9$) or (b) hPYY$_{3-36}$ ($n=9$) into the paraventricular nucleus. BIBP3226 (0.4 nmol) was mixed with [Leu^{31},Pro^{34}]NPY ($n=6$), hPYY$_{3-36}$ ($n=11$) or saline ($n=7$ in both experiments). Each group of rats was injected immediately before the test. Data are presented as the amount of food consumed (g) in 1 h test (mean$\pm$s.e.mean). $*P<0.05$, $**P<0.005$-significantly different from controls ($n=7$ and 11). $\#P<0.05$-significantly different from rats treated with NPY agonist alone (Scheffe's test).

Discussion

NPY is the most potent stimulant of feeding so far known and NPY mRNA is overexpressed in the hypothalamus in genetically obese animals (Kesteson *et al*., 1997). Several different NPY receptors have been identified and initially it was thought that the NPY Y_1 receptor mediates the orexigenic effect of NPY. Recently, it was suggested that a receptor subtype which is similar, but not identical to the NPY Y_1 receptor regulates food intake (Stanley *et al*., 1992). Following the cloning of the NPY Y_5 receptor (Gerald *et al*., 1996; Hu *et al*., 1996), which is now believed to be the 'feeding' receptor, contradictory data describing the effect of the selective NPY Y_1 receptor antagonist BIBP3226 have been published (Gerald *et al*., 1996; O'Shea *et al*., 1997) and the role of the NPY Y_1 receptor activation in feeding behaviour is unclear.

Our findings indicate that BIBP3226 reduces food intake in several feeding paradigms and are in agreement with previous findings supporting the involvement of the NPY Y_1 receptor in the regulation of food intake (Kanatani *et al*., 1996; O'Shea *et al*., 1997). In the present study BIBP3226 (10.0 nmol, i.c.v. and 0.4 nmol PVN) did not affect spontaneous food intake. Our experimental conditions did not allow the detection of the effects of BIBP3226 on macronutrient selections. Therefore, it is still possible that BIBP3226 may decrease basal carbohydrate ingestion, as demonstrated for another putative NPY Y_1 receptor antagonist, PYX-2 (Leibowitz *et al*., 1992). Testing was carried out near the end of the light period. It has been shown however, that the activity of NPY-ergic systems peaks during the dark period (McKibbin *et al*., 1991) and the maximal response to NPY following injection into the PVN occurs in the early portion of the dark period (Tempel & Leibowitz, 1990). Thus, it could be argued that BIBP3226 may be without effect when the activity of NPY-ergic neurotransmission is low. It was not possible to administer higher doses of BIBP3226 intracerebrally due to the low solubility of this compound. Hence, it can not be excluded that more potent and better soluble novel NPY Y_1 antagonists will reduce also spontaneous food intake.

Food deprivation increases NPY-like immunoreactivity and NPY release in the hypothalamus (Sahu *et al*., 1988; Calza *et al*., 1989; Beck *et al*., 1990; Yoshihara *et al*., 1996) and prepro-NPY mRNA in the arcuate nucleus (O'Shea & Gundlach, 1991). To investigate the effects of BIBP3226 in the conditions of increased NPY-ergic activity, the effects of BIBP3226 on food intake stimulated by fasting were studied. The food intake induced by fasting was reduced only by the highest dose of BIBP3226 (10.0 nmol) tested. This dose of BIBP3226 also reduced the food intake in another paradigm, namely the

NPY-induced food intake. Gerald *et al*. (1996) reported that BIBP3226 had no effect on NPY-induced food intake when measured 4 h after injection. However, antagonism of NPY (1.2 nmol)-induced feeding by BIBP3226 (60 nmol) was recently reported by others (O'Shea *et al*., 1997). These studies used the same dose of BIBP3226 as used by Gerald and colleagues (10.0 nmol) and show that if BIBP3226 is administered before NPY, the NPY-induced feeding is reduced at 0.5 and 4 h. Previously it has been shown that 10.0 nmol BIBP3226 has anxiogenic-like effects in an elevated plus-maze test (Kask *et al*., 1996; 1997). Thus, it is possible that the ability of BIBP3226 to reduce fasting and NPY-stimulated food intake after i.c.v. administration is an unspecific, anxiety-related effect. If the ability of BIBP3226 to suppress food intake is caused by increased anxiety, it could be possible to antagonize this with established anxiolytic drugs. In our laboratory a low dose of diazepam (0.5 mg kg^{-1}) antagonized the anxiogenic-like effect of BIBP3226 (Kask *et al*., 1996) and the same dose also counteracted the neophobia caused by the noradrenergic neurotoxin, DSP-4 (Harro *et al*., 1995). Benzodiazepines themselves have the propensity to increase food intake, but this usually occurs at high doses or when highly palatable diets are used (Cooper, 1980; Higgs & Cooper, 1996). In this study diazepam antagonized the effect of BIBP3226 on NPY-induced food intake suggesting that the ability of BIBP3226 to reduce NPY-induced food intake may be explained by anxiogenic effects of BIBP3226. Such an interpretation could be questioned because of the aforementioned ability of benzodiazepines to increase food intake (this study and our unpublished findings). However, the increase in food intake after diazepam in the present study was small, occurred at 2 h and as such does not explain the complete reversal of the effects of BIBP3226 on NPY-induced food intake. Thus, it is possible, that after i.c.v. administration BIBP3226 suppresses the food intake by generating anxiety.

When *ad libitum* fed rats are offered sweet carbohydrate rich diets, some animals overeat and become obese (Sclafani *et al*., 1996; Levin & Dunn-Meynell, 1997). On the other hand, NPY selectively increases the ingestion of carbohydrates (Leibowitz & Alexander, 1991; Welch *et al*., 1994) and repeated injections of NPY into the PVN lead to increased weight gain mimicking the development of diet-induced obesity (Stanley *et al*., 1989). Although palatability induced ingestional response has not been used widely for pharmacological analysis, this relatively simple test based on diet-induced ingestional response may be useful for identifying the classes of drugs for treatment of human eating disorders. Palatable diets also have another advantage, they offer the possibility of distinguishing between the effects of drugs on appetite and hunger (Blundell & Thurlby, 1980). BIBP3226 reduced the food consumption in rats on first exposure to sweet carbohydrate rich mash. It could be argued that BIBP3226 did not reduce appetite, but enhanced neophobia towards novel food. However, this seems unlikely, as BIBP3226 also reduced the mash consumption in rats which were trained to eat sweetened mash and readily consumed it. The effect of BIBP3226 was significant only at an 'anxiogenic' dose (10.0 nmol) and a lower dose (1.0 nmol) only tended to reduce mash intake. Although this lower dose of BIBP3226 was not anxiogenic in our previous study (Kask *et al*., 1996), this does not provide conclusive evidence that BIBP3226 can suppress the food intake without increasing anxiety. A mapping study was performed to find the brain site which mediates the anxiogenic effect of i.c.v. BIBP3226. BIBP3226 (1 nmol) microinjected into the PVN, central nucleus of amygdala or into vicinity of the locus coeruleus did not affect the behaviour of the rats in an elevated plus-maze test, whereas such treatment significantly enhanced neophobia after injection into the dorsal periaqueductal gray matter (Kask *et al*., 1998). Though elevated plus-maze and food intake experiments cannot be directly compared, the inactivity of intra-PVN BIBP3226 in this test of anxiety suggests that the PVN is a brain area where anxiogenic-like and food intake-inhibiting effects of BIBP3226 can be dissociated.

As in previous studies (Kalra *et al*., 1991; Stanley *et al*., 1992), [Leu^{31},Pro^{34}]NPY, microinjected into the PVN, increased food intake. This effect was completely blocked by BIBP3226. The ability of BIBP3226 to suppress [Leu^{31},-Pro^{34}]NPY-induced feeding is particularly interesting as BIBP3226 is inactive at the recently cloned NPY Y_5 receptor (Gerald *et al*., 1996), whereas [Leu^{31},Pro^{34}]NPY has some affinity also for this 'feeding' receptor. As the effect of [Leu^{31},Pro^{34}]NPY was blocked completely, probably only the NPY Y_1 receptors were activated by this dose of [Leu^{31},-Pro^{34}]NPY. These data suggest that [Leu^{31},Pro^{34}]NPY increases food intake by acting at the Y_1 receptors in the PVN. Peri-PVN injections of BIBP3226 efficiently reduced the mash consumption. Hence, BIBP3226 attenuated palatability-induced ingestional responses and our findings are well in line with the hypothesis, that the activation of the NPY Y_1 receptors increases the consumption of carbohydrates (Leibowitz and Alexander, 1991).

Feeding is an evolutionally shaped behaviour, which occurs only when there is no imminent danger from the environment. The NPY Y_1 receptors mediate the anxiolytic-like effects of NPY, and the PVN, which mediates hormonal reactions to stress (Sawchenko *et al*., 1992), receives nerve terminals from several NPY-containing brain areas involved in the processing of anxiogenic stimuli (Chronwall *et al*., 1985; Holets *et al*., 1988). Therefore, it can be hypothesized that the activation of the NPY Y_1 receptor has a permissive role and increase in food intake occurs only when both NPY Y_5 and Y_1 receptors are simultaneously activated. To test this hypothesis and the specificity of BIBP3226 effects for NPY Y_1 receptor mediated events, the effect of BIBP3226 on feeding induced by $hPYY_{3-36}$ was studied. PYY_{3-36}, which may be formed endogenously (Eberlein *et al*., 1989), has high affinity for NPY Y_2 and Y_5 receptors and is virtually inactive at Y_1 receptor (Gerald *et al*., 1996). In addition to the NPY Y_5 receptors (Gerald *et al*., 1996), low levels of Y_1 and Y_2 binding sites have been found in the PVN (Gehlert & Gackenheimer, 1997). Apparently, the NPY Y_2 receptors are not involved in the regulation of feeding directly, as the food intake is not increased by i.c.v. and intra-PVN administration of the NPY Y_2 agonist NPY_{13-36} even in high doses (Kalra *et al*., 1991; O'Shea *et al*., 1997). Therefore, $hPYY_{3-36}$ microinjected into the PVN may be used for the stimulation of the NPY Y_5 receptors in feeding studies. BIBP3226 did not affect the feeding induced by $hPYY_{3-36}$. This finding does not confirm our hypothesis and suggests that feeding induced by $hPYY_{3-36}$ is independent of Y_1 receptor activation. The inability of BIBP3226 to affect $hPYY_{3-36}$ induced feeding also rules out the possibility that BIBP3226 antagonized [Leu^{31},Pro^{34}]NPY-induced feeding by physico-chemical incompatibility (e.g. by damaging or changing the biological properties of the peptide before the agonist reached the receptors) which could occur theoretically when two compounds are administered as a mixture. Interestingly, BIBP3226 has also been shown to antagonize galanin- and noradrenaline-induced feeding (O'Shea *et al*., 1997). This suggests that NPY Y_1 receptor activation may be the final common pathway initiating feeding response, although physiological antagonism (anxiety) or non-specific effects of

BIBP3226 (toxicity) could be considered. The ineffectiveness of BIBP3226 to modify $hPYY_{3-36}$-induced feeding confirms that BIBP3226 is indeed a Y_1-selective antagonist and BIBP3226 does not suppress the feeding behaviour as a result of toxic effect on hypothalamic neurons. As both [Leu^{31},Pro^{34}]NPY and $hPYY_{3-36}$ increased food intake and only the effects of [Leu^{31},Pro^{34}]NPY were blocked by BIBP3226, it can be concluded that the two pharmacologically distinct NPY receptors (Y_1 and Y_5?) regulate food intake. Alternatively, BIBP3226 may also act at the novel unidentified 'feeding' receptor (O'Shea *et al*., 1997) which is not sensitive to $hPYY_{3-36}$. The reason for the existence of several NPY receptors in the hypothalamus mediating feeding response is presently unknown. It has been proposed that that Y_1 receptor activation increases carbohydrate ingestion, whereas Y_2 receptors reduce it (Leibowitz & Alexander, 1991). Such data are not available for the selective NPY Y_5 agonist $hPYY_{3-36}$ and the function of this receptor subtype in macronutrient selection remains unknown. Most recently, the inhibition of the NPY Y_5 synthesis by antisense oligonucleotides has been shown to inhibit spontaneous food intake in rats (Schaffhauser *et al*., 1997). The development and pharmacological evaluation of NPY Y_5 antagonists will clarify the role of NPY receptor subtypes in the regulation of feeding behaviour and food selection.

In conclusion, the results of the present study have shown that selective NPY Y_1 receptor antagonist BIBP3226 inhibits food intake induced by agents activating the NPY Y_1 receptor. The suppression of fasting-induced and palatability-induced ingestional response by BIBP3226 suggests that this compound also antagonized the effects of endogenous NPY, as both conditions are associated with increased NPY-ergic activity in the hypothalamus. These findings provide additional evidence for the involvement of the NPY Y_1 receptor in the regulation of food intake.

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References

AKABAYASHI, A., WAHLESTEDT, C., ALEXANDER, J.T. & LEIBOWITZ, S.F. (1994). Specific inhibition of endogenous neuropeptide Y synthesis in arcuate nucleus by antisense oligonucleotides suppresses feeding behavior and insulin secretion. *Brain Res. Mol. Brain Res.,* **21,** 55–61.

BALASUBRAMANIAM, A., SHERRIF, S., JOHNSON, M.E., PRADHAKARAN, M., HUANG, Y., FISHER, J.E. & CHANCE, W.T. (1994). [D-Trp^{32}] neuropeptide Y: a competitive antagonist in rat hypothalamus. *J. Med. Chem.,* **37,** 811–815.

BECK, B., JHANWAR-UNIYAL, M., BURLET, A., CHAPLEUR-CHATEAU, M., LEIBOWITZ, S.F. & BURLET, C. (1990). Rapid and localized alterations of neuropeptide Y (NPY) in discrete hypothalamic nuclei with feeding status. *Brain Res.,* **528,** 245–249.

BLUNDELL, J.E. & THURLBY, P.L. (1980). Experimental manipulations of eating: advances in animal models for studying anorectic agents. *Pharmacol. Ther.,* **34,** 349–401.

CALZA, L., GIARDINO, L., BATTISTINI, N., ZANNI, M., GALETTI, S., PROTOPAPA, F. & VELARDO, A. (1989). Increase of neuropeptide Y-like immunoreactivity in the paraventricular nucleus of fasting rats. *Neurosci. Lett.,* **104,** 99–104.

CHRONWALL, B.M., DIMAGGIO, D.A., MASSARI, V.J., PICKEL, V.M., RUGGIERO, D.A. & O'DONOHUE, T.L. (1985). The anatomy of neuropeptide-Y-containing neurons in rat brain. *Neuroscience,* **15,** 1159–1181.

COOPER, S.J. (1980). Benzodiazepines as appetite-enhancing compounds. *Appetite,* **1,** 7–19.

DOODS, H.N., WIELAND, H.A., ENGEL, W., EBERLEIN, W., WILLIM, K.-D., ENTZEROTH, M. & WIENEN, W. (1996). BIBP 3226, the first selective neuropeptide Y_1 receptor antagonist: a review of its pharmacological properties. *Regul. Peptides,* **65,** 71–77.

EBERLEIN, G.A., EYSSELEIN, V.E., SCHAEFFER, M., LAYER, P., GRANDT, D., GOEBELL, H., NIEBEL, W., DAVIS, M., LEE, T.D., & SHIVELY, J.E. (1989). A new molecular form of PYY: structural characterization of human PYY(3–36) and PYY(1–36). *Peptides,* **10,** 797–803.

EVA, C., KEINÄNEN, K., MONYER, K., SEEBURG, P. & SPRENGEL, R. (1990). Molecular cloning of a novel G protein-coupled receptor that may belong to the neuropeptide receptor family. *FEBS Lett.,* **271,** 81–84.

GEHLERT, D.R. & GACKENHEIMER, S.L. (1997). Differential distribution of neuropeptide Y Y_1 and Y_2 receptors in rat and guinea-pig brains. *Neuroscience,* **76,** 215–224.

GERALD, C., WALKER, M.W., CRISCIONE, L., GUSTAFSON, E.L., BATZL-HARTMANN, C., SMITH, K.E., VAYSSE, P., DURKIN, M.M., LAZ, T.M., LINEMEYER, D.L., SCHAFFHAUSER, A.O., WHITEBREAD, S., HOFBAUER, K.G., TABER, R.I., BRANCHEK, T.A. & WEINSHANK, R.L. (1996). A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature,* **382,** 168–171.

GRUNDEMAR, L., WAHLESTEDT, C. & REIS, D.J. (1991). Neuropeptide Y acts at an atypical receptor to evoke cardiovascular depression and to inhibit glutamate responsiveness in the brainstem. *J. Pharmacol. Exp. Ther.,* **258,** 633–638.

HARRO, J., ORELAND, L., VASAR, E. & BRADWEJN, J. (1995). Impaired exploratory behaviour after DSP-4 treatment in rats: implications for the increased anxiety after noradrenergic denervation. *Eur. Neuropsychopharmacol.,* **5,** 447–455.

HEILIG, M. (1995). Antisense inhibition of neuropeptide Y (NPY)-Y_1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regul. Peptides,* **59,** 201–205.

HIGGS, S. & COOPER, S.J. (1996). Increased food intake following injection of the benzodiazepine receptor agonist midazolam into the IVth ventricle. *Pharmacol. Biochem. Behav.,* **55,** 81–86.

HU, Y., BLOOMQUIST, B.T., CORNFIELD, L.J., DECARR, L.B., FLORES-RIVEROS, J.R., FRIEDMAN, L., JIANG, P., LEWIS-HIGGINS, L., SADLOWSKI, Y., SCHAEFFER, J., VELAZQUEZ, N. & MCCALEB, M.L. (1996). Identification of a novel hypothalamic neuropeptide Y receptor associated with feeding behavior. *J. Biol. Chem.,* **271,** 26315–26319.

HULSEY, M.G., PLESS, C.M., WHITE, B.D. & MARTIN, R.J. (1995). ICV administration of anti-NPY antisense oligonucleotide: effects on feeding behavior, body weight, peptide content and peptide release. *Regul. Peptides,* **59,** 207–214.

HOLETS, V.R., HÖKFELT, T., RÖKAEUS, Å., TERENIUS, L. & GOLDSTEIN, M. (1988). Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their projections to the spinal cord, cerecral cortex and hypothalamus. *Neuroscience,* **24,** 893–906.

KALRA, S.P., DUBE, M.G., FOURNIER, A. & KALRA, P.S. (1991). Structure-function analysis of stimulation of food intake by neuropeptide Y: effects of receptor agonists. *Physiol. Behav.,* **50,** 5–9.

KANATANI, A., ISHIHARA, A., ASAHI, S., TANAKA, T., OZAKI, S. & IHARA, M. (1996). Potent neuropeptide Y Y_1 receptor antagonist, 1229U91: blockade of neuropeptide Y-induced and physiological food intake. *Endocrinology,* **137,** 3177–3182.

KASK, A., RÅGO, L. & HARRO, J. (1998). Anxiogenic-like effect of the NPY Y_1 receptor antagonist BIBP3226 administered into the dorsal periaqueductal gray matter in rats. *Regul. Peptides,* in press.

KASK, A., RÅGO, L. & HARRO, J. (1997). α-helical CRF_{9-41} prevents anxiogenic-like effect of NPY Y_1 receptor antagonist BIBP3226 in rats. *Neuroreport,* **8,** 3645–3647.

KASK, A., RÅGO, L. & HARRO, J. (1996). Anxiogenic-like effect of the neuropeptide Y_1 receptor antagonist BIBP3226: antagonism with diazepam. *Eur. J. Pharmacol.,* **317,** R3–R4.

KESTERSON, R.A., HUSZAR, D., LYNCH, C.A., SIMERLY, S.B. & CONE, R.D. (1997). Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of agouti obesity syndrome. *Mol. Endocrinol.,* **11,** 630–637.

KRAUSE, J., EVA, C., SEEBURG, P.H. & SPRENGEL, R. (1992). Neuropeptide Y1 subtype pharmacology of a recombinantly expressed neuropeptide receptor. *Mol. Pharmacol.,* **41,** 817–821.

LEIBOWITZ, S.F. & ALEXANDER, J.T. (1991). Analysis of neuropeptide Y-induced feeding: dissociation of Y_1 and Y_2 receptor effects on natural meal patterns. *Peptides,* **12,** 1251–1260.

LEIBOWITZ, S.F., XUEREB, M. & KIM, T. (1992). Blockade of natural and NPY induced carbohydrate feeding by a receptor antagonist PYX-2. *Neuroreport,* **3,** 1023–1026.

LEVIN, B.E. & DUNN-MEYNELL, A.A. (1997). Dysregulation of arcuate nucleus preproneuropeptide Y mRNA in diet-induced obese rats. *Am. J. Physiol.,* **272,** R1365–R1370.

LOPEZ-VALPUESTA, F.J., NYCE, J.W. & MYERS, R.D. (1996). NPY-Y_1 receptor antisense injected centrally in rats causes hyperthermia and feeding. *Neuroreport,* **7,** 2781–2784.

LUNDELL, I., STATNICK, M.A., JOHNSON, D., SCHOBER, D.A., STARBÄCK, P., GEHLERT, D.R. & LARHAMMAR, D. (1996). The cloned rat pancreatic polypeptide receptor exhibits profound differences to the orthologous human receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 5111–5115.

MALMSTRÖM, R.E., BALMER, K.C. & LUNDBERG, J.M. (1997). The neuropeptide Y (NPY) Y_1 receptor antagonist BIBP 3226: equal effects on vascular responses to exogenous and endogenous NPY in the pig *in vivo*. *Br. J. Pharmacol.,* **121,** 595–603.

MATOS, F.F., GUSS, V. & KORPINEN, C. (1996). Effects of neuropeptide Y (NPY) and [D-Trp^{32}]NPY on monoamine and metabolite levels in dialysates from rat hypothalamus during feeding behavior. *Neuropeptides,* **30,** 391–398.

MCKIBBIN, P.E., ROGERS, P. & WILLIAMS, G. (1991). Increased neuropeptide-Y concentrations in the lateral hypothalamic area of the rat after the onset of darkness: possible relevance to the circadian periodicity of feeding behavior. *Life Sci.,* **48,** 2527–2533.

MICHEL, M.C., BECK-SICKINGER, A., COX, H., DOODS, H.N., HERZOG, H., LARHAMMAR, D., QUIRION, R., SCHWARTZ, T. & WESTFALL, T. (1998). XVI International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY and pancreatic polypeptide receptors. *Pharmacol. Rev.,* **50,** 143–150.

O'SHEA, D., MORGAN, D.G.A., MEERAN, K., EDWARDS, C.M.B., TURTON, M.D., CHOI, S.J., HEATH, M.M., GUNN, I., TAYLOR, G.M., HOWARD, J.K., BLOOM, C.I., SMALL, C.J., HADDO, O., MA, J.J., CALLINAN, W., SMITH, D.M., GHATEI, M.A. & BLOOM, S.R. (1997). Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* **138,** 198–202.

O'SHEA, R.D. & GUNDLACH, A.L. (1991). Preproneuropeptide Y messenger ribonucleic acid in the hypothalamic arcuate nucleus of the rat is increased by food deprivation or dehydration. *J. Neuroendocrinol.,* **3,** 11–14.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates,* 2nd edn. Academic Press: Sydney, p 242.

PALEA, S., CORSI, M., RIMLAND, J.M., TRIST, D.G. & RATTI, E. (1995). Failure of the putative neuropeptide Y antagonists, benextramine and PYX-2, to inhibit Y_2 receptors in rat isolated prostatic vas deferens. *Br. J. Pharmacol.,* **116,** 2401–2406.

PAEZ, X., NYCE, J.C. & MYERS, R.D. (1991). Differential feeding responses evoked in the rat by NPY and NPY_{1-27} injected intracerebroventricularly. *Pharmacol. Biochem. Behav.,* **38,** 379–384.

RUDOLF, K., EBERLEIN, W., ENGEL, W., WIELAND, H.A., WILLIM, K.D., ENTZEROTH, M., WIENEN, W., BECK-SICKINGER, A.G. & DOODS, H. (1994). The first potent and selective non-peptide neuropeptide Y Y_1 receptor antagonist: BIBP3226. *Eur. J. Pharmacol.,* **271,** R11–R13.

SAHU, A., KALRA, P.S. & KALRA, S.P. (1988). Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. *Peptides,* **9,** 83–86.

SAWCHENKO, P.E., CUNNINGHAM, E.T., BITTENCOURT, J.C. & CHAN, R.K.W. (1992). Aminergic and peptidergic pathways subserving the stress response. In *Stress: Neuroendocrine and Molecular Approaches* R. Kvetnansky, R. McCarty, & J. Axelrod (eds), Gordon and Breach Science, New York. pp. 15–27.

SCHAFFHAUSER, A.O., STRICKER-KRONGRAD, A., BRUNNER, L., CUMIN, F., GERALD, C., WHITEBREAD, S., CRISCIONE, L. & HOFBAUER, K.G. (1997). Inhibition of food intake by neuropeptide Y Y_5 receptor antisense oligodeoxynucleotides. *Diabetes,* **46,** 1792–1798.

SCLAFANI, A., LUCAS, F. & ACKROFF, K. (1996). The importance of taste and palatability in carbohydrate-induced overeating in rats. *Am. J. Physiol.,* **270,** R1197–R1202.

STANLEY, B.G. (1993). Neuropeptide Y in multiple hypothalamic sites controls eating behavior, endocrine, and autonomic systems for body energy balance. In *The Biology of Neuropeptide Y and Related Peptides* W.F. Colmers & C. Wahlestedt (eds), Humana Press Inc, Totowa. pp, 457–509.

STANLEY, B.G., MAGDALIN, W., SEIRAFI, A., NGUYEN, M.M. & LEIBOWITZ, S.F. (1992). Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y_1 receptor mediating this peptide's effect. *Peptides,* **13,** 581–587.

STANLEY, B.G., ANDERSON, K.C., GRAYSON, M.H. & LEIBOWITZ, S.F. (1989). Repeated hypothalamic stimulation with neuropeptide Y increases daily carbohydrate and fat intake and body weight gain in female rats. *Physiol. Behav.,* **46,** 173–177.

ST-PIERRE, S.-A., DUMONT, Y., NOUEL, D., HERZOG, H., HAMEL, E. & QUIRION, R. (1998). Preferential expression of the neuropeptide Y Y_1 over the Y_2 receptor subtype in cultured hippocampal neurones and cloning of the rat Y_2 receptor. *Br. J. Pharmacol.,* **123,** 183–194.

STRICKER-KRONGRAD, A., BECK, B. & BURLET, C. (1996). Enhanced feeding response to neuropeptide Y in hypothalamic neuropeptide Y-depleted rats. *Eur. J. Pharmacol.,* **295,** 27–34.

SWANSON, L. (1992). *Brain Maps: Structure of the Rat Brain,* New York, Elsevier.

TEMPEL, D.L. & LEIBOWITZ, S.F. (1990). Diurnal variations in the feeding responses to norepinephrine, neuropeptide Y and galanin in the PVN. *Brain Res. Bull.,* **25,** 821–825.

WELCH, C.C., GRACE, M.K., BILLINGTON, C.J. & LEVINE, A.S. (1994). Preference and diet type affect macronutrient selection after morphine, NPY, norepinephrine, and deprivation. *Am. J. Physiol.,* **266,** R426–R433.

WIELAND, H.A., WILLIM, K.-D., ENTZEROTH, M., WIENEN, W., RUDOLF, K., EBERLEIN, W., ENGEL, W. & DOODS, H.N. (1995). Subtype selectivity and antagonistic profile of the nonpeptide Y_1 receptor antagonist BIBP 3226. *J. Pharmacol. Exp. Ther.,* **275,** 143–149.

YOSHIHARA, T., HONMA, S., KATSUNO, Y. & HONMA, K. (1996). Dissociation of paraventricular NPY release and plasma corticosterone levels in rats under food deprivation. *Am. J. Physiol.,* **271,** E239–E245.

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