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Peptides 26 (2005) 1167–1175
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# NPY-induced feeding: pharmacological characterization using selective opioid antagonists and antisense probes in rats

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Received 14 December 2004; received in revised form 20 January 2005; accepted 24 January 2005
Available online 26 February 2005

**Abstract**

The ability of neuropeptide Y to potently stimulate food intake is dependent in part upon the functioning of μ and κ opioid receptors. The combined use of selective opioid antagonists directed against μ, δ or κ receptors and antisense probes directed against specific exons of the MOR-1, DOR-1, KOR-1 and KOR-3/ORL-1 opioid receptor genes has been successful in characterizing the precise receptor subpopulations mediating feeding elicited by opioid peptides and agonists as well as homeostatic challenges. The present study examined the dose-dependent (5–80 nmol) cerebroventricular actions of general and selective μ, δ, and $\kappa_1$ opioid receptor antagonists together with antisense probes directed against each of the four exons of the MOR-1 opioid receptor gene and each of the three exons of the DOR-1, KOR-1, and KOR-3/ORL-1 opioid receptor genes upon feeding elicited by cerebroventricular NPY (0.47 nmol, 2 ug). NPY-induced feeding was dose-dependently decreased and sometimes eliminated following pretreatment with general, μ, δ, and $\kappa_1$ opioid receptor antagonists. Moreover, NPY-induced feeding was significantly and markedly reduced by antisense probes directed against exons 1, 2, and 3 of the MOR-1 gene, exons 1 and 2 of the DOR-1 gene, exons 1, 2, and 3 of the KOR-1 gene, and exon 3 of the KOR-3/ORL-1 gene. Thus, whereas the opioid peptides, β-endorphin and dynorphin $A_{1–17}$ elicit feeding responses that are respectively more dependent upon μ and κ opioid receptors and their genes, the opioid mediation of NPY-induced feeding appears to involve all three major opioid receptor subtypes in a manner similar to that observed for feeding responses following glucoprivation or lipoprivation.


*Keywords:* μ opioid receptor; δ opioid receptor; κ opioid receptor; Naltrexone; β-Funaltrexamine; Nor-binaltorphamine; Naltrindole

## 1. Introduction

The ability to elucidate the roles of opioid receptor subtypes in the mediation of feeding behavior (see review: [8]) was first enhanced by the development of selective opioid receptor subtype antagonists directed against μ, δ, and κ receptors, and subsequently by the use of antisense (AS) probes to establish the relationship of the cloned receptors to opioid actions using sequences complementary to regions of specific exons of mRNA to down-regulate opioid receptor proteins [48]. The use of both of these in vivo pharmacological and molecular techniques allows for the collection of converging and complementary information about opioid-mediated roles in food intake following homeostatic challenges and administration of orexigenic agonists. Thus, food intake and body weight were reduced by both the μ-selective antagonist, β-funaltrexamine (BFNA) [5,67] and AS probes directed against each of the four exons of the MOR-1 gene [38]. Glucoprivic feeding elicited by 2-deoxy-D-glucose (2DG) is reduced by μ (BFNA) and $\kappa_1$ (nor-binaltorphamine (NBNI)) antagonists [3–5,31] as well as AS probes directed against

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doi:10.1016/j.peptides.2005.01.017

exons of the MOR-1 and KOR-1 genes [12]. Lipoprivic feeding elicited by mercaptoacetate was reduced by μ (BFNA), $\kappa_1$ (NBNI) and δ (naltrindole (NTI)) opioid receptor antagonists as well as AS probes directed against exons of the MOR-1, KOR-1, and DOR-1 genes [64]. However, whereas the pattern of $\kappa_1$ and δ opioid antagonist-induced reductions in feeding following food deprivation corresponded closely with the respective abilities of AS probes directed against exons of the KOR-1 and DOR-1 genes, the ability of μ opioid antagonists to reduce deprivation-induced intake in rats and mice was far more potent than AS probes directed against exons of the MOR-1 gene [22,23]. This approach also provides more detailed information about the receptor mechanisms mediating feeding elicited by opioid agonists. Feeding elicited by OFQ/$N_{1–13}$ is reduced by pretreatment with AS probes directed against each of the three exons of the KOR-3/ORL-1 gene [39]. Whereas feeding responses elicited by the μ-selective agonists, [D-Ala$^2$, N-Me-Phe$^4$, Gly-ol$^5$]-enkephalin (DAMGO), morphine and the active morphine metabolite, morphine-6β-glucuronide (M6G) are each effectively blocked by μ (BFNA) antagonism, feeding elicited by either DAMGO or morphine are blocked by AS probes directed against exons 1 and 4, but not exons 2 or 3 of the MOR-1 gene [40,41]. In contrast, M6G-induced feeding is blocked by AS probes directed against exons 2 and 3, but not exons 1 or 4 of the MOR-1 gene [40,41]. These effects were also observed in analgesic assays and are suggestive of actions of different splice variants or isoforms of the MOR-1 gene (for reviews, see [48,54]). Although μ, $\kappa_1$, and to a lesser degree, δ opioid antagonists significantly reduced βEND-induced feeding, AS probes directed against exons 1, 3 or 4 of the MOR-1 gene produced the most pronounced effects upon BEND-induced feeding, firmly implicating the μ opioid receptor in mediating this response [59]. Although $\kappa_1$ and to a lesser degree, μ and δ opioid antagonists significantly reduced DYN-induced feeding, AS probes directed against exon 1 or 2 of the KOR-1 or KOR-3/ORL-1 genes produced the most pronounced effects upon DYN-induced feeding, firmly implicating the κ opioid receptor in mediating this response [58]. This level of analysis has not yet been applied to non-opioid orexigenic peptide agonists.

Neuropeptide Y (NPY) is among the most potent central orexigenic peptides (see reviews: [30,37,43]), stimulating feeding and body weight gain following ventricular [13,44,53] and direct administration into the hypothalamic paraventricular nucleus (PVN: [7,60–62]). In contrast, administration of antagonists, antisera or AS ODN probes directed against NPY decrease food intake and weight gain [1,36,63]. Opioid involvement in feeding elicited by NPY was confirmed initially by the ability of the general opioid antagonist, naloxone to decrease the magnitude, but not the latency of NPY-induced feeding following systemic and ventricular administration [42,44,56]. Administration of naloxone into the medial, but not rostral or caudal nucleus tractus solitarius (NTS) blocked NPY-induced feeding elicited from the PVN [32,34]. Moreover, Ntx pretreatment into the central nucleus of the amygdala (CeA) decreased feeding elicited by PVN NPY [16]. Indeed within the PVN, NPY and naloxone produce additive increases in c-fos activity in the CeA [49]. Further, whereas DAMGO promotes intake of a dilute sucrose solution relative to chow, indicative of an opioid mediation of reward, NPY promotes intake of the chow relative to the dilute sucrose solution, indicative of NPY mediation of energy levels [18]. The opioid receptor subtypes involved in the mediation of NPY-induced feeding have been examined. Whereas cerebroventricular pretreatment with the μ opioid antagonist, BFNA or the $\kappa_1$ opioid antagonist, NBNI decreased feeding elicited by a 5 ug (1.17 nmol) dose of NPY, central administration of either the δ opioid antagonist, NTI or the κ opioid antagonist, GNTI failed to alter NPY-induced feeding [28,33]. To provide further analysis of these effects, the present study examined the dose-dependent actions of general (Ntx) and selective μ (BFNA), δ (NTI) and $\kappa_1$ (NBNI) opioid antagonists together with AS probes directed against each of the four exons of the MOR-1 gene and each of the three exons of the DOR-1, KOR-1 and KOR-3/ORL-1 genes upon feeding elicited by NPY.

## 2. Methods

### 2.1. Subjects and surgery

Adult male Sprague–Dawley rats (275–300 g; Charles River Laboratories, Wilmington, MA) were individually housed in suspended wire cages and maintained on a 12 h light:12 h dark cycle with rat chow pellets (Purina 5001 Rodent Diet, St. Louis, MO) in food bins and water available ad libitum. All animals were pretreated with chlorpromazine (3 mg/kg, i.p.) and were anesthetized with ketamine HCl (140 mg/kg, i.m.). A stainless steel guide cannula (22-gauge, Plastics One, Roanoke, VA) was implanted steroetaxically (Kopf Instruments, Tujunga, CA) into the left lateral ventricle using the following coordinates: incisor bar (+5 mm), 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture, and 3.6 mm from the top of the skull. Each cannula was secured to the skull by three anchor screws with dental acrylic. All animals were allowed at least 2 weeks to recover from stereotaxic surgery before behavioral testing began. After completion of behavioral testing, which took approximately 6 to 8 weeks for each animal, all rats were sacrificed with an overdose of anesthetic, and cannula placements were verified by visual inspection; all animals in the data analysis had cannula placements in the lateral ventricle.

### 2.2. Preliminary NPY dose–response curve

To confirm previously determined increases in food intake following NPY, and to select a dose of NPY that produced robust effects at the lowest concentration, a dose–response curve for NPY was created by testing animals from the lowest to highest doses. All behavioral testing was

conducted in the home cage between 2 and 8 h after the onset of the light cycle to minimize circadian effects on food intake. Rats were adapted to at least 4 days of baseline testing to eliminate any novelty-induced feeding responses elicited by placement of the pellets on the floor of the cage. It should be noted that intake during this phase of the light cycle is minimal as reflected by the low control values. In this and all subsequent protocols, before any experimental conditions, the food bins were removed from each cage and replaced with preweighed food pellets. Each intake value was measured by the weight (g) of the food pellets and adjusted by spillage that was collected on paper towels placed below the wire mesh cage. After baseline measurements, a group of six cannulated rats was assessed for food intake after 1, 2 and 4 h after microinjection of NPY (Peninsula Labs, Belmont, CA) at doses of 0, 0.12, 0.47, and 1.17 nmol (0, 0.5, 2.0, and 5.0 ug) administered at weekly intervals. All infusions were administered in a 2-ul volume of distilled water over 30 s through a stainless steel internal cannula (28-gauge, Plastics One) that extended 0.5–1.0 mm beyond the tip of the guide cannula, and which was connected to a Hamilton microsyringe by polyethylene tubing. After infusion, the internal cannula was removed and immediately replaced with a stainless steel dummy cannula (28-gauge, Plastics One) to prevent any effusion and to insure cannula patency between microinjection conditions. A repeated-measures analysis of variance revealed that significant differences in food intake were observed across injection conditions ($F(3,18) = 8.54$, $P < 0.001$) and across test times ($F(2,6) = 16.12$, $P < 0.0001$). Dose-dependent increases in food intake across the time course relative to vehicle occurred following the 0.12, 0.47, and 1.17 nmol NPY doses (Table 1). Although the 0.47 nmol dose of NPY produced a very consistent, but not the highest feeding response across the time course, it was chosen for the subsequent opioid antagonist and opioid AS probe studies because opioid AS effects have previously been observed for moderate, but not optimal doses of either 2DG-induced feeding [12,38] or DAMGO-induced feeding [41].

### 2.3. General and selective opioid antagonists, NPY, and food intake

All antagonists were administered in 2–5 ul volumes of distilled water to guarantee solubility of the compounds. All 19 cannulated rats in the four antagonist studies were initially assessed for food intake 1, 2, and 4 h after vehicle and after a NPY dose of 0.47 nmol to verify that all animals displayed feeding responses following the agonist. The animals were exposed to a maximum of four different antagonist treatments in counterbalanced orders with a 1-week interval between the treatments. Subgroups ($n = 6$) of rats, matched for NPY-induced intake, were tested at weekly intervals across the following antagonist pretreatment conditions paired with NPY: the general opioid antagonist, Ntx (Sigma-Aldrich, St. Louis, MO) at doses of 1.89, 7.56, 15.12 or 30.24 ug (5–80 nmol), the μ opioid antagonist, BFNA (Sigma-Aldrich) at doses of 2.45, 9.8 or 19.6 ug (5–40 nmol), the δ opioid antagonist, NTI (Sigma-Aldrich) at doses of 2.55, 10.2 or 20.4 ug (5–40 nmol) or the $\kappa_1$ opioid antagonist, NBNI (Sigma-Aldrich) at doses of 3.65, 14.6 or 29.2 ug (5–40 nmol). The pretreatment time intervals of 1 h (Ntx, NTI, NBNI) and 24 h (BFNA) between antagonist and agonist treatments reflected the respective peak and selective actions of the opioid antagonists [5,50–52] and was consistent with our previous studies evaluating antagonist effects upon feeding elicited by BEND and DYN $A_{1–17}$ [58,59]. Food intake was assessed 1, 2, and 4 h following the second (NPY) injection.

### 2.4. AS ODN probes, NPY, and food intake

As described previously, all 44 cannulated rats in the AS studies were initially assessed for food intake 1, 2, and 4 h after vehicle and after a NPY dose of 0.47 nmol to verify that all animals displayed feeding responses following the agonist. All AS probes were administered in 10 ug doses dissolved in 5 ul volumes of 0.9% normal saline based upon their previously determined effectiveness in agonist-induced feeding studies [39–41,58,59] without producing nonspecific effects (for review, see [48]). All phosphodiester oligodeoxynucleotides (Midland Certified Reagent, Midland, TX) were purified in our (G.W. Pasternak and G.C. Rossi) laboratories, and the identified locations of the AS probes were based on the different opioid receptor gene sequences listed in GenBank (Table 2). The opioid AS sequences directed against the individual exons of the MOR-1, DOR-1, KOR-1 or KOR-3/ORL-1 opioid receptor genes used in the present study in rats are based upon the rat clone (for review, see [54]). During each 6-day test phase, rats received microinjections of their particular AS probes on days 1, 3, and 5 as previously described [39–41,58,59]; this time course of treatment both down-regulates the synthesis of new receptors and permits turnover of existing receptors (for review, see [48]). Rats were exposed to a maximum of two different AS treatments with a minimal 2-week interval between AS treatments. Subgroups ($n = 6$ each) of the 44 rats tested in this paradigm were assigned to the following conditions by matching increased

Table 1
Alterations in food intake following NPY

| | Dose (nmol) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vehicle | | | 0.12 | | | 0.47 | | | 1.17 | | |
| Time (h) | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 |
| Intake (g) | 0.2 | 1.1 | 2.4 | 2.4 | 2.4 | 3.2 | 4.1 | 6.0 | 6.4 | 6.4 | 7.2 | 7.9 |

Table 2
Sequence of antisense oligodeoxynucleotides

| Probe | Sequence |
|---|---|
| MOR-1 opioid receptor clone | |
| Exon 1 AS | CGC CCC AGC CTC TTC CTC T |
| Exon 2 AS | TTG GTG GCA GTC TTC ATT TTG G |
| Exon 3 AS | TGA GCA GGT TCT CCC AGT ACC A |
| Exon 4 AS | GGG CAA TGG AGC AGT TTC TG |
| DOR-1 opioid receptor clone | |
| Exon 1 AS | TGT CCG TCT CCA CCG TGC |
| Exon 2 AS | ATC AAG TAC TTG GCG CTC TG |
| Exon 3 AS | AAC ACG CAG ATC TTG GTC AC |
| KOR-1 opioid receptor clone | |
| Exon 1 AS | GCT GCT GAT CCT CTG AGC CCA |
| Exon 2 AS | CCA AAG CAT CTG CCA AAG CCA |
| Exon 3 AS | GGC GCA GGA TCA TCA GGG TGT |
| KOR-3/ORL-1 opioid receptor clone | |
| Exon 1 AS | GGG GCA GGA AAG AGG GAC TCC |
| Exon 2 AS | GAC GAG GCA GTT CCC CAG GA |
| Exon 3 AS | GGG CTG TGC AGA AGC CGA GA |

food intake after NPY (0.47 nmol) administration: AS probes directed against exons 1, 2, 3 or 4 of the MOR-1 gene; directed against exons 1, 2 or 3 of the DOR-1 gene; directed against exons 1, 2 or 3 of the KOR-1 gene; or directed against exons 1, 2 or 3 of the KOR-3/ORL-1 gene. Twenty-four hours after the last AS treatment (day 6), all rats received NPY (0.47 nmol), and food intake was assessed after 1, 2, and 4 h. Consistent with our observations in previous studies (e.g., [58,59]), neither the opioid antagonists nor the antisense probes produced any adverse effects on the general health of the animals.

### 2.5. Statistics

To determine significant effects in the antagonist and AS paradigms, separate two-way repeated-measures analyses of variance were performed with the treatment conditions (i.e., different doses of a specific antagonist or various exons of a specific AS probe) as one variable and test times as the second variable. Tukey comparisons ($P < 0.05$) were used to determine individual significant agonist effects relative to vehicle treatment, and to determine individual significant antagonist or AS probe effects relative to NPY treatment.

## 3. Results

### 3.1. Opioid antagonist effects upon NPY-induced feeding

Significant differences in food intake were observed among treatment conditions ($F(14,101) = 4.18$, $P < 0.0001$), across test times ($F(2,202) = 71.93$, $P < 0.0001$) and for the interaction between conditions and times ($F(28,202) = 2.14$, $P < 0.014$). NPY at a dose of 0.47 nmol produced a robust

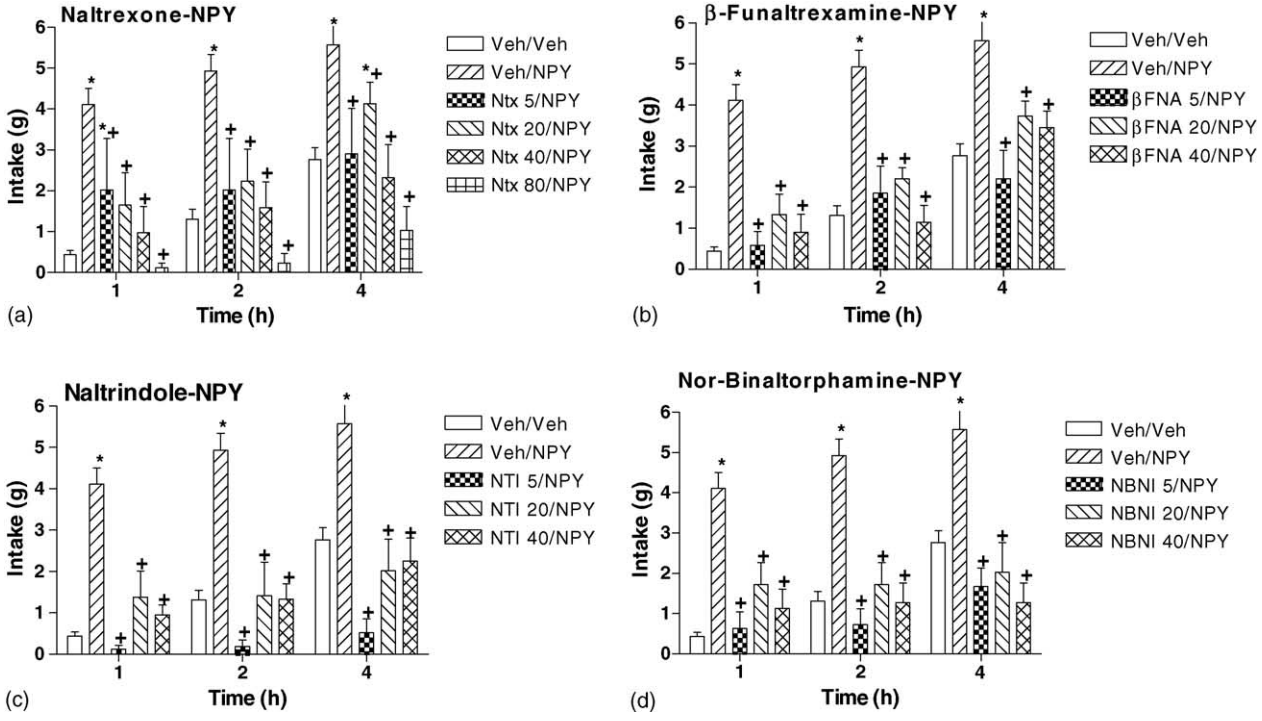


Fig. 1. Alterations (mean ± S.E.M.) in food intake (g) after i.c.v. administration of either vehicle (Veh), neuropeptide Y (NPY: 0.47 nmol) or NPY after ventricular pretreatment with the general opioid antagonist, naltrexone (Ntx) at doses of either 5, 20, 40 or 80 nmol, the μ opioid antagonist, β-funaltrexamine (BFNA) at doses of 5, 20 or 40 nmol, the δ opioid antagonist, naltrindole (NTI) at doses of 5, 20 or 40 nmol, or the $\kappa_1$ opioid antagonist, nor-binaltorphamine (NBNI) at doses of 5, 20 or 40 nmol. Significant increases in food intake by NPY relative to vehicle treatment are denoted by asterisks (*), and significant decreases in NPY-induced feeding by opioid antagonists are denoted by crosses (+).

increase in feeding after 1, 2, and 4 h relative to vehicle treatment (Fig. 1). The magnitude of NPY-induced feeding was significantly reduced (50–80%) across the 4 h time course by the 5 and 20 nmol Ntx doses, and was abolished across the 4 h time course by the 40 and 80 nmol doses of the general opioid antagonist (Fig. 1, upper left panel). The magnitude of NPY-induced feeding was significantly reduced (65–75%) across the 4 h time course by the 20 nmol BFNA dose, and was abolished across the 4 h time course by the 5 and 40 nmol doses of the μ opioid antagonist (Fig. 1, upper right panel). The magnitude of NPY-induced feeding was significantly reduced (75%) across the 4 h time course by the 20 nmol NTI dose, and was abolished across the 4 h time course by the 5 and 40 nmol doses of the δ opioid antagonist (Fig. 1, lower left panel). The magnitude of NPY-induced feeding was significantly reduced (65–88%) across the 4 h time course by the 20 nmol NBNI dose, and was abolished across the 4 h time course by the 5 and 40 nmol doses of the $\kappa_1$ opioid antagonist (Fig. 1, lower right panel).

### 3.2. Opioid AS probe effects upon NPY-induced feeding

Significant differences in food intake were observed among treatment conditions ($F(14,151) = 3.13$, $P < 0.0003$), across test times ($F(2,302) = 49.56$, $P < 0.0001$), but not for the interaction between conditions and times ($F(28,302) = 0.78$, ns). NPY at a dose of 0.47 nmol produced a robust increase in feeding after 1, 2, and 4 h relative to vehicle treatment in this paradigm (Fig. 2) comparable to that observed in the previous protocol. The magnitude of NPY-induced feeding was respectively abolished (2–4 h) or significantly reduced (1 h) by AS probes directed against exons 1 (80–95%), and 2 (75–87%) of the MOR-1 gene (Fig. 2, upper left panel). Whereas the AS probe directed against exon 3 of the MOR-1 gene significantly reduced (42–54%) NPY-induced feeding over the 4 h time course, the AS probe directed against exon 4 of the MOR-1 gene was ineffective (Fig. 2, upper left panel). The magnitude of NPY-induced feeding was significantly reduced across the 4 h time course by AS probes directed against exons 1 (39–58%) and 2 (72–84%) of the DOR-1 gene; the AS probe directed against exon 3 was ineffective (Fig. 2, upper right panel). The magnitude of NPY-induced feeding was significantly reduced across the 4 h time course by AS probes against the KOR-1 gene with the probe directed against exon 1 (82–87%) producing more robust effects than exon 2 (42–59%); the AS probe directed against exon 3 produced effects only after 1 h (Fig. 2, lower left panel). Although NPY-induced feeding was significantly reduced across the 4 h time course by AS probes against the KOR-3/ORL-1 gene, the magnitude of effect of the probe against exon 3 (39–49%) was generally less pronounced. Whereas the AS probe directed against exon 1 was transient (4 h), the AS probe directed against exon 2 was ineffective (Fig. 2, lower right panel).

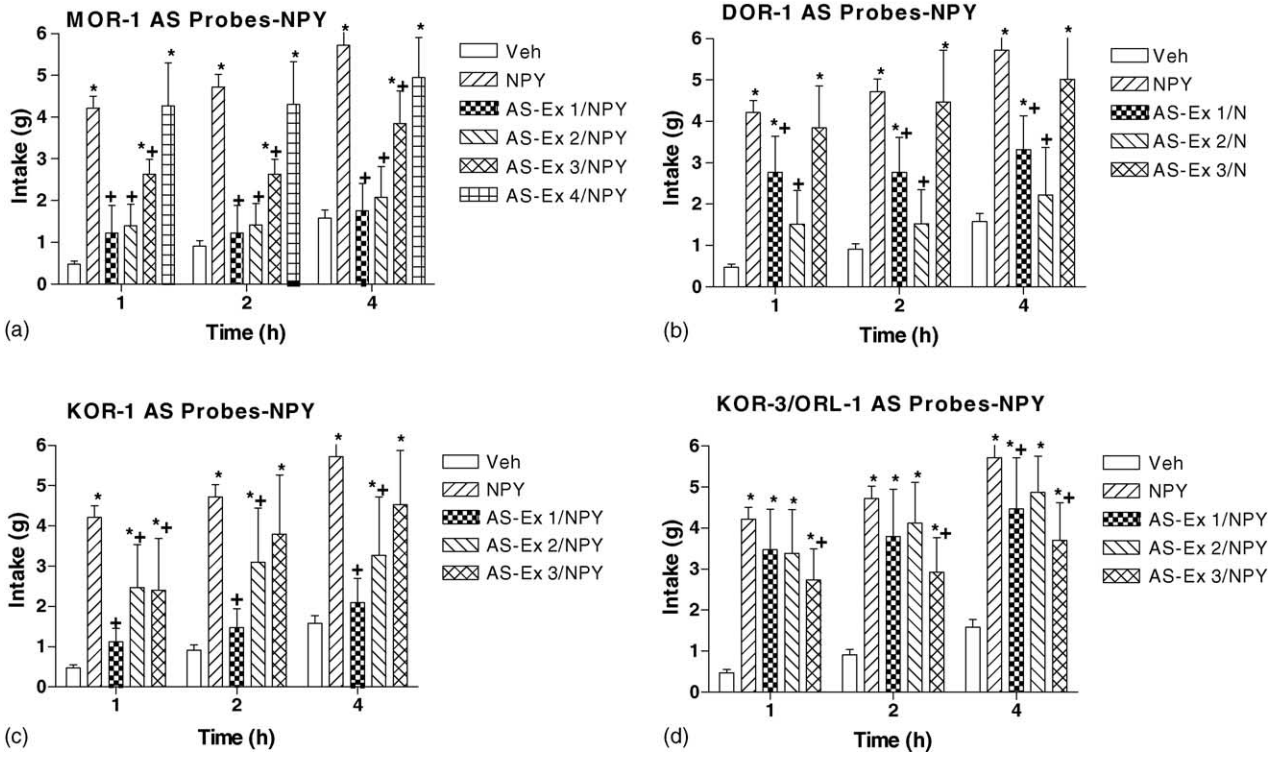


Fig. 2. Alterations (mean ± S.E.M.) in food intake (g) after i.c.v. administration of either vehicle (Veh), neuropeptide Y (NPY: 0.47 nmol) or NPY after ventricular pretreatment with antisense (AS) probes (10 ug) directed against exons (Ex) 1, 2, 3 or 4 of the MOR-1 opioid receptor gene or against exons (Ex) 1, 2 or 3 of the DOR-1, KOR-1 or KOR-3/ORL-1 opioid receptor genes. Significant increases in food intake by NPY relative to vehicle treatment are denoted by asterisks (*), and significant decreases in NPY-induced feeding by opioid AS probes are denoted by crosses (+).

## 4. Discussion

The strong and robust feeding response elicited by NPY was significantly reduced by general opioid receptor pretreatment, consistent with previous findings [16,32,34,42,44,56]. Furthermore, the ability of the selective μ and κ opioid antagonists, BFNA and NBNI to reduce NPY-induced feeding is also consistent with a previous report [33]. Whereas this previous study failed to observe reductions in NPY-induced feeding following δ opioid antagonism with NTI, the present study observed significant decreases in NPY-induced feeding following NTI. This difference can be explained by the use of a lower (0.47 nmol), but still very effective orexigenic dose of NPY than the dose (1.17 nmol) employed previously [33]. The effects of the AS probes directed against the MOR-1, DOR-1, KOR-1, and KOR-3/ORL-1 genes provided highly consistent and converging lines of evidence concerning opioid mediation of NPY-induced feeding. Thus, like the μ antagonist, BFNA, AS probes directed especially against exons 1 and 2 of the MOR-1 gene virtually eliminated NPY-induced feeding with AS probes directed against exons 3 and 4 producing respectively smaller magnitudes of effects. Similar to effects of the δ antagonist, NTI, AS probes directed against exons 1 and 2 of the DOR-1 gene significantly reduced NPY-induced feeding. An AS probe directed against exon 3 of the DOR-1 gene was ineffective. Finally, like the $\kappa_1$ antagonist, NBNI, the AS probe directed against exon 1 of the KOR-1 gene significantly reduced NPY-induced feeding. AS probes directed against exons 2 and 3 of the KOR-1 gene and exons 1, 2, and 3 of the KOR-3/ORL-1 gene produced smaller magnitudes of effects. One caveat regarding the present study was that NPY-induced feeding per se was not reassessed following antagonist and antisense treatments. Therefore, one cannot definitively rule out that the effects were due to Tachyphylaxis. However, as the remainder of the discussion indicates, we have observed a number of instances of antagonist- and antisense-specific effects as a function of the orexigenic agonist employed.

These data indicate that feeding elicited by NPY is similar to that of opioid peptides and opiate agonists and metabolites in their sensitivity to opioid antagonists and AS probes (Table 3). Thus, μ antagonists decrease NPY-induced feeding to the same degree as feeding responses to BEND, DYN, morphine, DAMGO and M6G (e.g., [40,41,58,59]). The sensitivity of NPY-induced feeding to the AS probe directed against exon 1 of the MOR-1 gene is shared by feeding responses elicited by BEND, DYN, morphine and DAMGO, but not M6G. The sensitivity of NPY-induced feeding to the AS probe directed against exon 2 of the MOR-1 gene is shared by feeding responses elicited by M6G and to a lesser degree, BEND, but not morphine, DAMGO or DYN. Whereas the AS probe directed against exon 3 of the MOR-1 gene significantly and potently reduces feeding induced by BEND and M6G, it produces more modest effects upon NPY-induced feeding and fails to affect feeding following DYN or DAMGO. Whereas the AS probe directed against exon 4 of the MOR-1 gene potently affects feeding following BEND and DAMGO, it produces minimal (NPY) or no (M6G) effects on other agonist-induced feeding responses. The MOR-1 AS exon-specific effects of DAMGO and morphine (exons 1 and 4, not 2 or 3) and M6G (exons 2 and 3, not 1 or 4) on feeding and analgesic responses suggest that different isoforms of the MOR-1 gene exist (e.g., [46,47]). However, like BEND, the sensitivity of NPY-induced feeding to multiple MOR-1 AS probes suggests that this response is mediated by multiple coding regions of the MOR-1 gene.

Feeding elicited by NPY is most markedly reduced by an AS probe against exon 2 of the DOR-1 gene, but is also reduced by an AS probe directed against exon 1. The latter probe abolished feeding elicited by the δ opioid agonist, Deltorphin, produced less pronounced effects upon feeding elicited by BEND and DYN, and failed to affect M6G-induced feeding [40,58,59]. Feeding elicited by NPY is most markedly reduced by an AS probe against exon 1 of the KOR-1 gene, but is also reduced by AS probes directed against exons 2 and 3 of the KOR-1 gene and exons 1 and 3 of the KOR-3/ORL-1 gene. The AS probe against exon 1 of the KOR-1 gene abolished feeding elicited by either DYN or the $\kappa_1$ opioid agonist, U50488H, but failed to affect feeding elicited by BEND and M6G [40,58,59]. The modest effects upon NPY-induced feeding of AS probes directed against exons 1 and 3 of the KOR-3/ORL-1 gene stand in contrast to their potent effects upon feeding induced by DYN and OFQ/$N_{1–13}$ and the absence of effects upon feeding induced by BEND and M6G [39,40,58,59]. Thus, whereas feeding elicited by BEND

Table 3
Comparison of opioid antagonist and AS probe effects upon feeding responses elicited by NPY and the opioid peptides, BEND and DYN

| Condition | NPY | BEND[a] | DYN[b] |
|---|---|---|---|
| BFNA | ↓↓ | ↓↓ | ↓↓ |
| MOR-1 | | | |
| AS Ex 1 | ↓↓ | ↓↓ | ↓ |
| AS Ex 2 | ↓↓ | ↓ | None |
| AS Ex 3 | ↓ | ↓↓ | None |
| AS Ex 4 | None | ↓↓ | None |
| NTI | ↓↓ | ↓↓ | ↓↓ |
| DOR-1 | | | |
| AS Ex 1 | ↓ | ↓ | ↓ |
| AS Ex 2 | ↓↓ | None | None |
| AS Ex 3 | None | None | None |
| NBNI | ↓↓ | ↓↓ | ↓↓ |
| KOR-1 | | | |
| AS Ex 1 | ↓↓ | None | ↓↓ |
| AS Ex 2 | ↓ | None | ↓↓ |
| AS Ex 3 | ↓ | None | None |
| KOR-3 | | | |
| AS Ex 1 | Min | None | ↓↓ |
| AS Ex 2 | None | None | ↓ |
| AS Ex 3 | ↓ | None | None |

*Note*. ↓↓: >70% reduction; ↓: ∼50% reduction; Min: ∼20–30% reduction; None: not significant.
[a] Data derived from Ref. [59].
[b] Data derived from Ref. [58].

appears more selective to μ/MOR-1 effects [59] and whereas feeding elicited by DYN appears more selective to κ/KOR-1 effects [58], the opioid mediation of NPY-induced feeding appears to involve all three major opioid receptor subtypes.

The ability of multiple opioid receptor subtypes to modulate NPY-induced feeding is similar to the involvement of μ, δ, and κ receptors in lipoprivic (mercaptoacetate)-induced feeding using antagonist and AS approaches [64], the involvement of μ and κ receptors in both glucoprivic (2DG)-induced and food deprivation-induced feeding using an antagonist approach [3–5], of μ, κ, and δ receptors in 2DG-induced feeding using an AS approach [12], and of κ and to a lesser degree μ and δ receptors in deprivation-induced feeding using an AS approach [22,23]. Feeding responses elicited by NPY have been related to energy homeostasis relative to palatability given its induction of chow intake relative to the dilute sucrose intake [18]. The striking similarities of opioid antagonist and AS effects upon feeding responses induced by NPY and 2DG provide further support for their previously established inter-relationship such that 2DG increases NPY mRNA levels in the arcuate nucleus [2,57], and NPY levels are inversely related to administered glucose levels [68]. However, NPY, like food deprivation, is far more effective in increasing the "break point" for food pellet reinforcement relative to either 2DG or insulin [27].

Where and how might NPY and the opioid system interact with respect to neural circuits modulating ingestion? NPY, co-expressed with the orexigenic agouti gene-related peptide (AGRP) in the arcuate nucleus projects to a number of hypothalamic areas, including the PVN, ventromedial nucleus and lateral hypothalamic-perifornical area (e.g., [6,9,26]). NPY neurons share reciprocal connections with neural systems that inhibit feeding, notably the central pro-opiomelanocortin (POMC) melanocortin system in the arcuate nucleus (see reviews: [29,69]), and suppress their activity through GABA co-localization [14]. Whereas α-melanocyte stimulating hormone (α-MSH) and its analog, MTII inhibit food intake through melanocortin (MC-3R and MC-4R) receptors [10,15,17,20,65,66], BEND, the endogenous POMC opioid peptide, stimulates food intake (e.g., [19,35]) through μ receptors [59]. Both α-MSH and MTII completely suppress the orexigenic effects of NPY (e.g., [71]). As indicated in the present and previous [33] studies indicating opioid receptor subtype involvement in NPY-induced feeding, opioid interactions are also observed for food-induced modulation by α-MSH and AGRP. Thus, AGRP-induced feeding, acting via MC-3R and MC-4R receptor antagonism (e.g., [24,55,70]), is blocked by either systemic or central naloxone [25,45] or combined μ and κ antagonist treatment into the third ventricle [11]. Whereas the μ opioid antagonist, BFNA decreased feeding induced by the MC-3R/4R receptor antagonist, SHU-9119, MTII decreased BEND-induced feeding [21]. Therefore, it would appear that endogenous opioid peptides acting on local μ, κ, and δ receptors in the PVN, arcuate and other hypothalamic nuclei interact with NPY and other orexigenic hypothalamic peptides (AGRP, orexin, melanin-concentrating hormone: see review: [37]) to stimulate feeding under a variety of homeostatic and palatable conditions.

## Acknowledgements

This research was supported in part by Professional Staff Congress/City University of New York Grants 65285-34 and 66325-35 (R.J.B.) and National Institute on Drug Abuse Grants DA07274 (G.W.P.), DA00220 (G.W.P.) and DA13997 (Y.X.P.). Y. Israel performed this work as his Honors Thesis in the CUNY Honors College at Queens College.

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