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Opioid Receptor Involvement in Food Deprivation-Induced Feeding: Evaluation of Selective Antagonist and Antisense Oligodeoxynucleotide Probe Effects in Mice and Rats

M. M. Hadjimarkou, A. Singh, Y. Kandov, Y. Israel, Y.-X. Pan, G. C. Rossi, G. W. Pasternak, and R. J. Bodnar

Department of Psychology and Neuropsychology Doctoral Sub-Program, Queens College, City University of New York, Flushing, New York (M.M.H., A.S., Y.K., Y.I., R.J.B.); Laboratory of Molecular Neuropharmacology, Memorial Sloan-Kettering Cancer Center, New York, New York (Y.-X.P., G.C.R., G.W.P.); and Department of Psychology, C. W. Post College, Long Island University, Brookville, New York (G.C.R.)

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ABSTRACT

Central administration of general and selective opioid receptor subtype antagonists in the rat has revealed a substantial role for μ, a moderate role for κ, and a minimal role for δ receptors in the mediation of deprivation-induced feeding. Antisense probes directed against the κ opioid receptor (KOP), nociceptin opioid receptor (NOP), and δ opioid receptor (DOP) genes in rats result in reductions similar to κ and δ antagonists, whereas antisense probes directed against the μ opioid receptor (MOP) gene produced modest reductions relative to μ antagonists, suggesting that isoforms of the MOP gene may mediate deprivation-induced feeding. Since these isoforms were initially identified in mice, the present study compared the effects of general and selective opioid receptor antagonists on deprivation-induced feeding in rats and mice and antisense probes directed against exons of the MOP, DOP, KOP, and NOP genes on deprivation-induced feeding in the mouse. Food-deprived (12 and 24 h) rats and mice displayed similar profiles of reductions in deprivation-induced feeding following general, μ, and κ opioid antagonists. In contrast, mice, but not rats, displayed reductions in deprivation-induced intake following δ antagonism as well as DOP antisense probes, suggesting a species-specific role for the δ receptor. Antisense probes directed against the KOP and NOP genes also reduced deprivation-induced intake in mice in a manner similar to κ antagonism. However, the significant reductions in deprivation-induced feeding following antisense probes directed against either exons 2, 4, 7, 8, or 13 of the MOP gene were modest compared with μ antagonism, suggesting a role for multiple μ-mediated mechanisms.

Endogenous opioid peptides and receptor systems are intimately involved in the mediation of food intake (see review, Bodnar, 2004). Systemic and central administration of general opioid antagonists significantly reduce deprivation-induced feeding in rats and mice (e.g., Holtzman, 1974; Brown and Holtzman, 1979; Frenk and Rogers, 1979; Cooper, 1980). Opioid receptor-selective antagonists differentially alter deprivation-induced feeding in rats, such that ventricular pretreatment with either μ- or μ_1-selective antagonists produce potent reductions (50–75%) in deprivation-induced intake, effects equal in magnitude to general opioid antagonism (Simone et al., 1985; Arjune et al., 1990; Levine et al., 1991; Koch and Bodnar, 1994). In contrast, κ-selective antagonists marginally (~30%) reduce deprivation-induced feeding (Levine et al., 1990; Koch and Bodnar, 1994), whereas δ-selective antagonists fail to alter this response (Arjune et al., 1991; Koch and Bodnar, 1994). Therefore, it appears that opioid receptor mediation of deprivation-induced feeding in rats occurs primarily through the μ, secondarily through the κ, and minimally through the δ receptor.

AS ODN probes (Pasternak and Standifer, 1995; Rossi and

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ABBREVIATIONS: AS ODN, antisense oligodeoxynucleotide(s); MOP, μ opioid receptor; MOR-1, μ opioid receptor clone; DOP, δ opioid receptor; DOR-1, δ opioid receptor clone; KOP, κ opioid receptor; KOR-1, κ opioid receptor clone; NOP, nociceptin opioid receptor; KOR-3/ORL-1, κ3/orphan receptor-like opioid receptor clone; 2DG, 2-deoxy-D-glucose; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; M6G, morphine-6-glucuronide; βFNA, β-funaltrexamine; NTI, naltrindole; NBNI, nor-binaltorphamine.

Pasternak, 1997) have been used in ingestive studies to correlate the molecular biology of the cloned opioid receptors [MOP (MOR-1: 4 exons), DOP (DOR-1: 3 exons), KOP (KOR-1: 3 exons), NOP (KOR-3/ORL-1: 3 exons); see review, Uhl et al., 1994] with their in vivo functional effects and to provide converging evidence to studies using opioid receptor subtype antagonists. Our laboratory (Hadjimarkou et al., 2003b) demonstrated that deprivation-induced feeding in rats was maximally reduced by an AS ODN probe directed against exon 2 of the KOP gene, an effect complementary to κ antagonist effects, whereas probes directed against exons 2, 3, or 4 of the MOP gene, exon 1 of the DOP gene, and exon 1 of the NOP gene resulted in modest reductions in deprivation-induced intake. The modest reductions observed in the food-deprived rats after MOP antisense administration clearly diverged from the potent reductions observed after μ-selective antagonists. This stands in marked contrast to converging effects between μ antagonists and MOP antisense probes in feeding responses elicited by 2DG-induced glucoprivation, mercaptoacetate-induced lipoprivation, the μ agonist, DAMGO, the morphine metabolite, M6G, and the opioid peptides β-endorphin and dynorphin A(1–17) (see review, Bodnar, 2004). Given that there is only one identified MOP gene (e.g., Wang et al., 1993), the modest actions of MOP AS ODN probes upon deprivation-induced feeding suggest that the μ receptor antagonist actions upon deprivation-induced feeding may involve splice variants of the MOP gene that have been identified in the mouse (Bare et al., 1994; Zimprich et al., 1995; Pan et al., 1999, 2000, 2001; Pasternak and Pan, 2000; Pasternak, 2001).

Thus, to evaluate whether isoforms of the MOP (MOR-1) gene are responsible for μ-mediated actions, AS ODN probes directed against these additional exons (5a, 6, 7, 8, 9, 10, 12, and 13) identified in the mouse can be used. However, although mice and rats display the same pattern of naloxone-induced inhibition of deprivation-induced feeding (e.g., Holtzman, 1974; Brown and Holtzman, 1979; Frenk and Rogers, 1979; Cooper, 1980), the effects of selective opioid receptor subtype antagonists upon deprivation-induced feeding have only been analyzed in rats. Thus, a systematic comparison of selective opioid antagonist effects upon deprivation-induced feeding in the mouse and the rat is warranted. Given the size differences of the two species, it is conceivable that 24 h of deprivation may constitute a greater homeostatic stressor upon the mouse than the rat. Therefore, the first two experiments examined the effects of general and selective opioid antagonists upon food intake and body weight changes (24–48 h) following 24 or 12 h of food deprivation in mice. The third experiment examined the effects of general and selective opioid antagonists upon food intake and body weight changes following 24 h of food deprivation in rats. The fourth experiment examined whether AS ODN probes directed against each of the following exons of MOP (exons 1, 2, 3, 4, 5a, 6, 7, 8, 9, 10, 12, and 13), DOP (exons 1, 2, and 3), KOP (exons 1, 2, and 3), and NOP (exons 1 and 2) genes altered food intake and body weight changes following 24 h of food deprivation in mice.

Materials and Methods

Subjects and Surgery: Mice. All procedures and protocols described in this study were approved by the Queens College Institutional Animal Care and Use Committee (Protocol 38: 3/02–3/05). Male CD-1 mice (25–30 g; Charles River Laboratories, Inc., Wilmington, MA) were housed in groups (four to five mice) in clear polyethylene cages and were maintained on a 12-h light/dark cycle with water and standard mouse chow pellets (Purina Lab Diet 5015) available ad libitum until behavioral testing took place (see *Procedures*). In performing ventricular injections, mice were exposed to an isoflurane and oxygen combination for approximately 2 to 3 min until full anesthesia was observed. A midline incision along the sagittal suture allowed visualization of the sutures, and a freehand microinjection was administered in the lateral ventricle at 2 mm anterior to the lambda suture and 3 to 3.5 mm lateral to the midline suture through an internal cannula (28-gauge) that extended 2 mm below the surface of the skull. This procedure was periodically verified in control mice using Luxol fast blue injections to visualize entry into the ventricles. Mice received one and only one experimental condition.

Subjects and Surgery: Rats. Male albino Sprague-Dawley rats (300–350 g; Charles River Laboratories, Inc.) were housed individually in suspended wire mesh cages and were maintained on a 12-h light/dark cycle with water and standard rat chow pellets (Purina Lab Diet 5001) available ad libitum. All animals were acclimated to the vivarium facility for at least 2 weeks before they underwent stereotaxic surgery. Each rat was pretreated with chlorpromazine (3 mg/kg i.p.) and anesthetized with ketamine HCl (120 mg/kg i.m.). A stainless steel guide cannula (22-gauge; Plastics One, Roanoke, VA) was implanted stereotaxically (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. The cannula was secured to the skull by dental acrylic (Plastics One) and three anchor screws. All rats were gently handled during the surgical recovery period lasting 2 further weeks before behavioral testing began. After completion of behavioral testing, all rats were sacrificed with an overdose of anesthetic (Euthasol; Del Marva Laboratories, Henry Schein, Long Island, NY), and the cannula placements were histologically verified.

Opioid Antagonists (Experiments 1–3). Naltrexone, βFNA, NBNI, and NTI were purchased from Sigma-Aldrich (St. Louis, MO) and were dissolved in distilled water. All ventricular antagonist microinjections were administered in 5-μl volumes over 30 s through a stainless internal cannula (28-gauge; Plastics One) connected by polyethylene tubing to a Hamilton microsyringe. In the rat, the internal cannula was removed and replaced by a dummy cannula to prevent efflux. Systemic naltrexone injections were administered subcutaneously at 1 ml/kg b.wt. in the rat and at 10 ml/kg b.wt. in the mouse.

Opioid Antisense Probes (Experiment 4). The identified locations of the AS ODN probes are based upon the different opioid receptor gene sequences listed in GenBank (Table 1). All phosphodiester AS ODN probes (Midland Certified Reagent, Midland, TX) were purified by ethanol precipitation in one of our laboratories (G.W.P., Y.-X.P.) and dissolved (0.9% normal saline) to a 5-μl concentration. The AS ODN sequences (19–22 bases long) directed against the individual exons of either the MOP, DOP, KOP, or NOP opioid receptor genes are specific to the mouse clone (see review, Rossi and Pasternak, 1997). All ventricular AS ODN microinjections were administered in the mouse in 2-μl volumes (10 μg dose) over 30 to 60 s. During each test phase, mice received three microinjections of their particular AS ODN probe on days 1, 3, and 5 with testing commencing on day 6 as previously described (e.g., Leventhal et al., 1997, 1998b; Burdick et al., 1998; Stein et al., 2000; Silva et al., 2001, 2002; Hadjimarkou et al., 2003b); this time course of treatment is presumed to down-regulate existing receptors as well as the synthesis of new receptors (see reviews, Pasternak and Standifer, 1995; Rossi and Pasternak, 1997).

Opioid Antagonist Testing Procedures in Mice: Experiment 1. Following acclimation of the mice in individual cages equipped

TABLE 1
Opioid antisense oligodeoxynucleotide probes targeted against the mouse genome
An AS ODN directed against exon 3 of the NOP gene for the mouse was unavailable and not used in this study.

Probe	Sequence
MOP gene	
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
Exon 5a AS	GGG GTT GGC ACC AGC ATT AGG TAC TC
Exon 6 AS	GGC TCA AAG ACA AGG GAC AGG TCA
Exon 7 AS	CCT GTA AAG ACT GTG GCA CCG C
Exon 8 AS	GGG CCA TCA TCA GGA AGA AGG
Exon 9 AS	GAA AGG CAT CTT CCC TCT CGC T
Exon 10 AS	CTT GCT GCC TTC GTA AGG ACC TGG
Exon 12 AS	GGA CAA AGT GAA CAT CAG AGC CAG
Exon 13 AS	CAG GAA AAG AAT GGA CAG AGG
DOP gene	
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
KOP gene	
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
NOP gene	
Exon 1 AS	GGG GCA GGA AAG AGG GAC TCC
Exon 2 AS	GAC GAG GCA GTT CCC CAG GA

with metal floor grids, mice were weighed and assigned to a 4-day treatment paradigm. In the first control phase, food was removed from each animal's cage at 2 h into the light cycle (day 1), and the animals were food-deprived for 24 h. Food was reintroduced on day 2 by placing preweighed standard chow pellets at the floor of each cage. Intake was measured (±0.1 g) by weighing the pellets placed on the grid floor and adjusting for spillage collected by brown paper towels beneath the metal grid at 0.5, 1, 2, and 4 h thereafter. Food intake, adjusted for spillage, was also measured 24 (day 3) and 48 (day 4) h following food reintroduction. Body weight was monitored on all 4 days so that deprivation-induced weight loss and food reintroduction-induced weight recovery could be evaluated. The second experimental phase began 2 weeks later after it was determined that all mice regained their original body weight and demonstrated normal intake. Subgroups of mice, matched for 24-h deprivation-induced food intake, received either systemic naltrexone at doses of 0.1 ($n = 5$), 1 ($n = 5$), or 5 ($n = 10$) mg/kg, ventricular naltrexone at doses of 10 ($n = 8$) or 50 ($n = 10$) μg, ventricular βFNA at doses of 5 ($n = 8$) or 20 ($n = 9$) μg, ventricular NBNI at doses of 5 ($n = 10$) or 20 ($n = 9$) μg, or ventricular NTI at doses of 5 ($n = 9$) or 20 ($n = 9$) μg. Antagonist treatments preceded reintroduction of food to deprived mice by a time interval based on the previously determined peak effects of the antagonists (0.5 h for naltrexone, 1 h for NBNI and NTI, and 24 h for βFNA; see review, Bodnar, 2004). Systemic naltrexone injections were administered subcutaneously in a 10 ml/kg volume. All mice in the second phase were exposed to the identical paradigm described for the first phase. Mice received one and only one experimental condition.

Opioid Antagonist Testing Procedures in Mice: Experiment 2. New groups of mice were exposed to the same paradigm described in experiment 1 with the following exceptions. Although mice in experiment 1 were food deprived at about 2 h into the light cycle and remained deprived for 24 h subsequently, the baseline and experimental phases of experiment 2 were conducted such that the mice were food-deprived at the onset of the dark cycle (day 1) for 12 h so that food was again reintroduced at the onset of the light cycle on day 2. Food intake was again assessed after 0.5, 1, 2, 4, 24, and 48 h. In the second experimental phase, subgroups of mice matched for 12-h deprivation-induced food intake, received either systemic naltrexone (5 mg/kg, $n = 9$), ventricular naltrexone (50 μg, $n = 10$), ventricular βFNA (20 μg, $n = 8$), ventricular NBNI (20 μg, $n = 10$), or ventricular NTI (20 μg, $n = 9$).

Opioid Antagonist Testing Procedures in Rats: Experiment 3. Following recovery from surgery and approximately 1 month after arrival in the vivarium, food intake and body weight of 20 acclimated rats were assessed over 48 h to verify normal feeding responses. The rats underwent the following food deprivation paradigm four times over a 10-week period in which all rats were allowed 2 weeks between conditions to fully recover their normal body weight and normal food intake. In the first week, all 20 rats received a vehicle (5 μl of normal saline) ventricular microinjection 30 min prior to food reintroduction. During the 4th, 7th, and 10th weeks, subgroups of five rats each received the following injections: systemic naltrexone at doses of either 0.1 ($n = 5$) or 1 ($n = 5$) mg/kg, ventricular naltrexone at doses of either 10 ($n = 6$) or 20 ($n = 5$) μg, and βFNA, NBNI, and NTI at doses of either 5 or 20 μg, respectively. The order of the antagonist conditions and doses was counterbalanced across the three tests, and the rats were matched for deprivation-induced intake values under vehicle control conditions.

In each condition, rats were weighed on day 1 (predeprivation weight), food-deprived with water available for 24 h, and then reweighed on day 2 (postdeprivation weight) to assess deprivation-induced weight loss. Intake was measured (±0.1 g) by weighing standard chow pellets before placing them on the grid floor and adjusting for spillage collected by brown paper towels placed underneath the grid at 0.5, 1, 2, and 4 h following the food reintroduction. Food intake was also measured 24 (day 3) and 48 (day 4) h thereafter. Body weight was monitored on all 4 days so that deprivation-induced weight loss and food reintroduction-induced weight recovery could be evaluated.

AS ODN Testing Procedures in Mice: Experiment 4. After acclimating the mice to individual housing in metal grid floor cages, they entered the first phase of testing described above (experiment 1) for 24 h of food deprivation. The second experimental phase took place 2 weeks later and lasted 8 days. On days 1, 3, and 5 at 2 to 4 h into the light cycle, subgroups of seven to eight mice received a microinjection of 1 of the 12 MOR-1 AS ODN probes (directed against coding exons 1, 2, 3, 4, 5a, 6, 7, 8, 9, 10, 12, or 13), the DOP gene AS ODN probes (directed against coding exons 1, 2, or 3), the KOP gene AS ODN probes (directed against coding exons 1, 2, or 3), or the NOP gene AS ODN probes (directed against coding exons 1 or 2) (Table 1). Following the third microinjection on day 5, the animals were food-deprived for 24 h with water available ad libitum. On day 6, food was reintroduced, and short-term cumulative intake was assessed after 0.5, 1, 2, and 4 h. Longer-term intake was assessed on days 7 (24 h) and 8 (48 h). Body weight was monitored throughout the paradigm as noted in experiment 1.

Statistics. Randomized-block two-way analyses of variance were performed in each of the four experiments on short-term cumulative food intake data with the between-subject variable of vehicle and the different antagonist or AS ODN conditions, the within-subject variable of postdeprivation intake time (0.5, 1, 2, and 4 h), and for the interaction between condition and time. Tukey comparisons ($P < 0.05$) were used to assess significant antagonist or AS ODN effects relative to vehicle control treatment. Randomized-block two-way analyses of variance were also performed in each of the four experiments on longer-term cumulative food intake data with the between-subject variable of vehicle and the different antagonist or AS ODN conditions, the within-subject variable of postdeprivation intake time (24 and 48 h), and for the interaction between condition and time. Deprivation-induced weight loss scores were assessed for each animal by subtracting the postdeprivation weight from its corresponding predeprivation weight, and recovery-induced weight gain scores were assessed for each animal by subtracting the recovery weight from its corresponding postdeprivation weight. Separate one-way analyses of variance were performed on deprivation-induced weight loss and recovery-induced weight gain in each of the four experiments for each antagonist or AS ODN treatment relative to

vehicle control values with Tukey comparisons ($P < 0.05$) indicating individual significant effects relative to vehicle control treatment.

Results

Selective Opioid Receptor Antagonism and Deprivation (24 h)-Induced Feeding in the Mouse

Significant differences in short-term (0.5–4 h) intake were observed among drug conditions [$F(11,1023) = 266.45$, $P < 0.0001$], across test times [$F(3,279) = 24978.91$, $P < 0.0001$], and for the interaction between conditions and times [$F(33,3069) = 232.90$, $P < 0.0001$]. Systemic administration of the general opioid antagonist naltrexone significantly and dose dependently reduced short-term deprivation-induced feeding at the 0.1 (0.5–2 h), 1 (0.5–4 h), and 5 (0.5–4 h) mg/kg doses (Fig. 1A). Correspondingly, short-term deprivation-induced feeding was significantly reduced across the 4-h time course following ventricular naltrexone doses of 10 and 50 μg (Fig. 1C). Moreover, short-term deprivation-induced feeding was significantly reduced across the 4-h time course by both the 5- and 20-μg doses of the μ antagonist, βFNA (Fig. 2A), the κ antagonist, NBNI (Fig. 2C), and the δ antagonist, NTI (Fig. 2E).

Significant differences in longer-term (24–48 h) intake were observed among drug conditions [$F(11,1001) = 517.40$, $P < 0.0001$], across test times [$F(1,91) = 1307.26$, $P < 0.0001$], and for the interaction between conditions and times [$F(11,1001) = 298.35$, $P < 0.0001$]. Systemic naltrexone administration produced modest (11–12%), although significant, dose-dependent (1–5 mg/kg) reductions in longer-term deprivation-induced feeding after 24, but not after 48 h. Intake after 48 h was actually significantly increased following the lowest (0.1 mg/kg) naltrexone dose (Fig. 1B). Correspondingly, longer-term deprivation-induced feeding was significantly reduced after 24 h following both ventricular naltrexone doses. Intake after 48 h was, respectively, significantly decreased and increased following the low and high naltrexone doses (Fig. 1D). Moreover, longer-term deprivation-induced feeding was significantly reduced after 24 and 48 h following both NBNI doses (Fig. 2D) and after 24 h following both doses of either βFNA (Fig. 2B) or NTI (Fig. 2F). Intake after 48 h was significantly increased following the lower NTI dose (Fig. 2F).

Although significant differences in deprivation-induced body weight loss occurred among drug groups [$F(11, 172) = 2.93$, $P < 0.001$] in mice, all of the antagonists failed to differ from control treatment in the magnitude of body weight loss (Table 2). Significant differences in body weight recovery following deprivation were observed among drug conditions [$F(11,1001) = 699.16$, $P < 0.0001$], across test times [$F(1,91) = 393.21$, $P < 0.0001$], and for

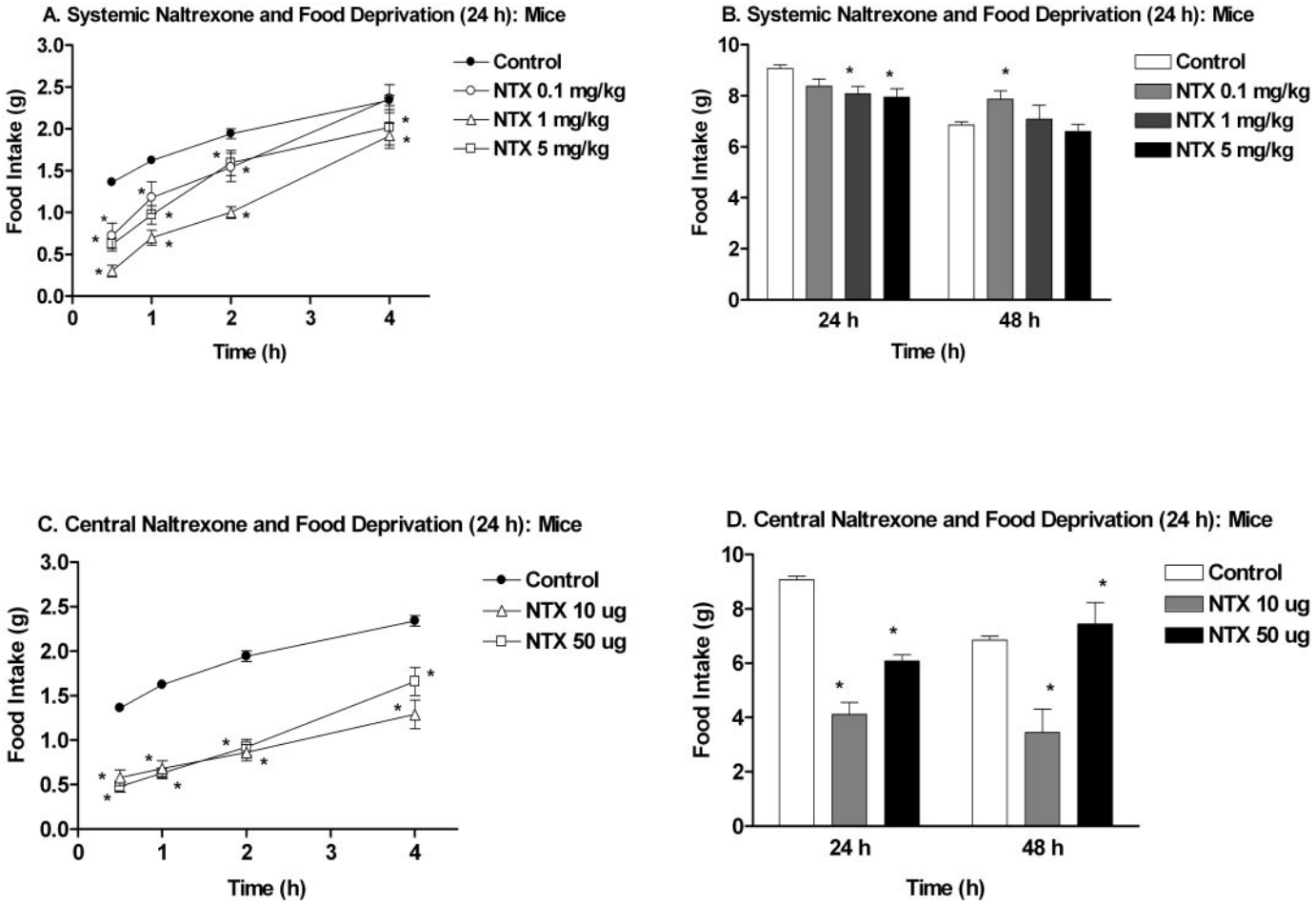

Fig. 1. Alterations in short-term (0.5–4 h; panels A and C) and longer-term (24–48 h; panels B and D) cumulative intake (mean ± S.E.M.) following systemic (panels A and B) or ventricular (panels C and D) administration of naltrexone in mice previously deprived of food for 24 h. The asterisks denote significant differences (Tukey comparisons, $P < 0.05$) from corresponding vehicle control conditions.

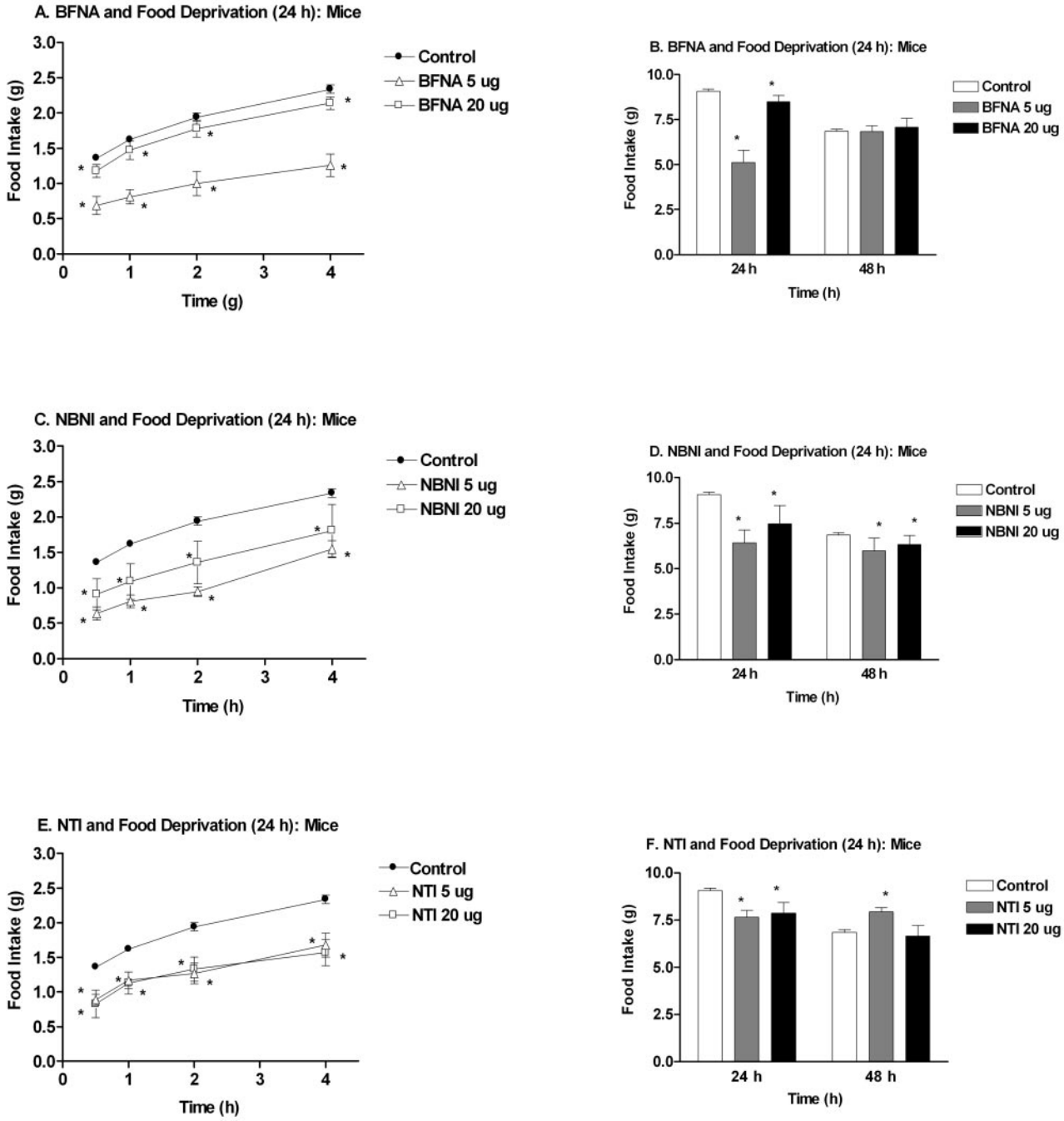

Fig. 2. Alterations in short-term (0.5–4 h; panels A, C, and E) and longer-term (24–48 h; panels B, D, and F) cumulative intake following ventricular administration of selective μ (βFNA; panels A and B), κ (NBNI; panels C and D), and δ (NTI; panels E and F) opioid receptor subtype antagonists in mice previously deprived of food for 24 h. The asterisks denote significant differences (Tukey comparisons, $P < 0.05$) from corresponding vehicle control conditions.

the interaction between conditions and times [$F(11,1001) = 155.90$, $P < 0.0001$]. Systemic naltrexone administration significantly reduced body weight recovery after 24 and 48 h following the 1 mg/kg dose, whereas ventricular naltrexone significantly reduced body weight recovery following the 10 (24–48 h)- and 50 (24 h)-μg doses (Table 2). Body weight recovery was significantly reduced following the 5 (24–48 h)-μg dose of βFNA, the 5 (24–48 h)- and 20 (24–48 h)-μg doses of NBNI, and the 5 (24 h)- and 20 (24–48 h)-μg doses of NTI (Table 2).

Selective Opioid Receptor Antagonism and Deprivation (12 h)-Induced Feeding in the Mouse

Significant differences in short-term (0.5–4 h) intake were observed among drug conditions [$F(5,220) = 169.53$, $P < 0.0001$], across test times [$F(3,132) = 1213.94$, $P < 0.0001$], and for the interaction between conditions and times [$F(15,660) = 36.58$, $P < 0.0001$]. Both systemic and ventricular naltrexone administration significantly reduced short-term deprivation-induced feeding across the time course (Fig. 3A). Correspondingly, short-term deprivation-induced

TABLE 2
Body weight loss and recovery in 24-h food-deprived mice treated with general and selective opioid receptor antagonists

Treatment	Body Weight Loss Mean (±S.E.M.)	Body Weight Recovery Mean (±S.E.M.)	
		24 h Post	48 h Post
	g		
Control	−5.35 (0.13)	5.32 (0.16)	5.78 (0.19)
Systemic naltrexone, s.c.			
0.1 mg/kg	−5.18 (0.24)	5.14 (0.53)	5.62 (0.53)
1.0 mg/kg	−4.28 (0.19)	3.82 (0.36)*	2.24 (1.79)*
5.0 mg/kg	−5.13 (0.53)	5.27 (0.54)	5.81 (0.48)
Central naltrexone, i.c.v.			
10 μg	−4.67 (0.75)	−0.89 (0.62)*	−1.05 (1.14)*
50 μg	−5.77 (0.23)	4.66 (0.31)*	5.87 (0.29)
βFNA			
5 μg	−5.58 (0.43)	2.89 (0.80)*	3.99 (0.47)*
20 μg	−6.57 (0.31)	6.06 (0.48)	6.31 (0.62)
NBNI			
5 μg	−4.57 (0.27)	2.53 (0.70)*	2.73 (1.20)*
20 μg	−4.84 (0.60)	3.52 (0.64)*	4.23 (0.88)*
NTI			
5 μg	−4.91 (0.28)	3.70 (0.41)*	5.28 (0.39)
20 μg	−3.73 (0.72)	4.00 (0.84)*	3.42 (3.33)*

*Significant reduction relative to corresponding control value.

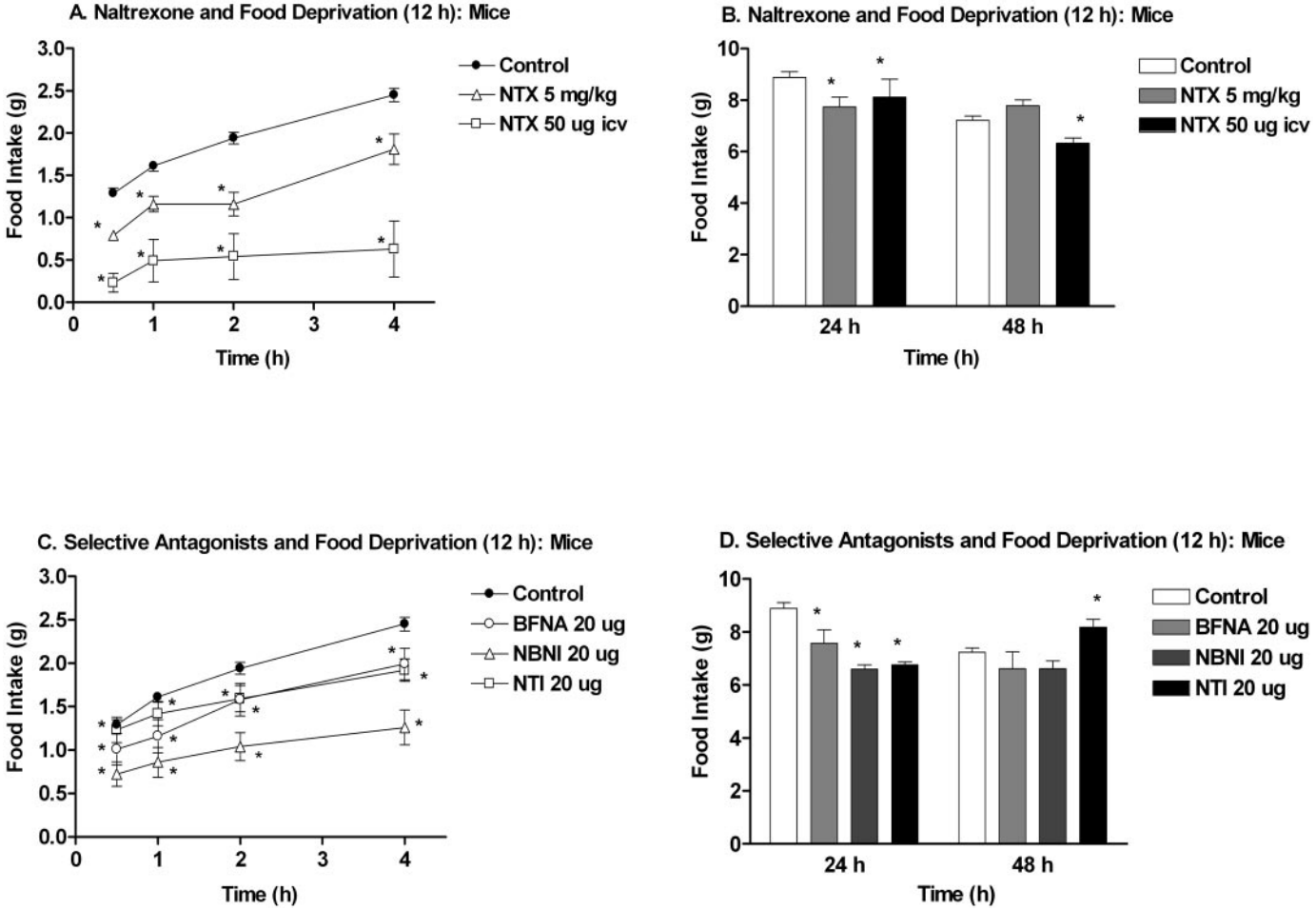

Fig. 3. Alterations in short-term (0.5–4 h; panels A and C) and longer-term (24–48 h; panels B and D) cumulative intake following administration of systemic and central naltrexone (panels A and B), and central selective μ (βFNA), κ (NBNI), and δ (NTI) opioid receptor subtype antagonists (panels C and D) in mice previously deprived of food for 12 h (dark cycle). The asterisks denote significant difefrences (Tukey comparisons, $P < 0.05$) from corresponding vehicle control conditions.

feeding was significantly reduced across the time course following βFNA, NBNI, and NTI pretreatment (Fig. 3C). Significant differences in longer-term (24–48 h) intake were observed among drug conditions [$F(5,220) = 30.57$, $P < 0.0001$], across test times [$F(1,44) = 242.92$, $P < 0.0001$], and for the interaction between conditions and times [$F(5,220) =$

128.50, $P < 0.0001$]. Longer-term deprivation-induced intake was significantly reduced by systemic (24 h) and ventricular (24–48 h) naltrexone (Fig. 3B), as well as by βFNA (24 h), NBNI (24 h), and NTI (24 h) pretreatment (Fig. 3D). Intake after 48 h was significantly increased following NTI (Fig. 3D). Although significant differences in deprivation-induced body weight loss failed to occur among drug groups [$F(5,85) = 0.86$, n.s., Table 3], significant differences in body weight recovery following deprivation were observed among drug conditions [$F(5,220) = 18.37$, $P < 0.0001$], across test times [$F(1,44) = 552.94$, $P < 0.0001$], and for the interaction between conditions and times [$F(5,220) = 56.08$, $P < 0.0001$]. Body weight recovery was significantly slowed by systemic (24 h), but not ventricular, naltrexone (Table 3). Body weight recovery was significantly reduced following βFNA (24–48 h) and NBNI (24–48 h) and significantly increased by NTI (48 h) (Table 3).

Selective Opioid Receptor Antagonism and Deprivation (24 h)-Induced Feeding in the Rat

Significant differences in short-term (0.5–4 h) intake were observed among drug conditions [$F(11,209) = 45.69$, $P < 0.0001$], across test times [$F(3,57) = 1078.53$, $P < 0.0001$], and for the interaction between conditions and times [$F(33,627) = 13.03$, $P < 0.0001$]. Whereas short-term deprivation-induced feeding was significantly reduced across the time course by all systemic naltrexone doses (Fig. 4A), it was dose dependently reduced by the low (0.5 h) and high (0.5–4 h) ventricular naltrexone doses (Fig. 4C). Whereas both βFNA doses significantly reduced short-term deprivation-induced feeding across the time course (Fig. 5A), it was dose dependently reduced by the low (1 h) and high (0.5–4 h) NBNI doses (Fig. 5C). In contrast, NTI failed to alter short-term deprivation-induced intake in the rat (Fig. 5E).

Significant differences in longer-term (24–48 h) intake were observed among drug conditions [$F(11,209) = 50.37$, $P < 0.001$] and for the interaction between conditions and times [$F(11,209) = 46.34$, $P < 0.001$], but not for test times [$F(1,19) = 0.41$, n.s.]. Whereas systemic naltrexone (1 mg/kg) only transiently (48 h) reduced longer-term deprivation-induced feeding (Fig. 4B), both ventricular naltrexone doses decreased longer-term deprivation-induced feeding after 24 and 48 h (Fig. 4D). Longer-term deprivation-induced intake was significantly reduced after 24 and 48 h following both βFNA doses (Fig. 5B) and the low NBNI dose (Fig. 5D), as well as after 24 h by the high doses of NBNI (Fig. 5D) and NTI (Fig. 5F).

The significant differences in deprivation-induced body weight loss occurred among drug groups [$F(11,65) = 2.41$, $P < 0.014$] indicated that weight loss was significantly greater in the NTI (5 μg) subgroup of rats before they received their low NTI dose (Table 4). Significant differences in body weight recovery following deprivation were observed among drug conditions [$F(11,209) = 67.22$, $P < 0.0001$], across test times [$F(1,19) = 755.10$, $P < 0.0001$], and for the interaction between conditions and times [$F(11,209) = 25.70$, $P < 0.0001$]. Both systemic and ventricular naltrexone administration significantly reduced body weight recovery after 24 and 48 h following all tested doses (Table 4). Moreover, both doses of μ, κ, and δ antagonists also significantly reduced body weight recovery after 24 and 48 h (Table 4).

Opioid Receptor Antisense Probes and Deprivation (24 h)-Induced Feeding in the Mouse

MOR-1 AS Effects on Intake. Significant differences in short-term (0.5–4 h) intake were observed among control and MOR-1 AS ODN conditions [$F(12,1032) = 124.16$, $P < 0.0001$], across test times [$F(3,258) = 13321.76$, $P < 0.0001$], and for the interaction between conditions and times [$F(36,3096) = 65.06$, $P < 0.0001$]. Short-term deprivation-induced intake was significantly and consistently reduced by AS ODN probes directed against coding exons 2 (2–4 h), 4 (0.5–4 h), 7 (1–4 h), 8 (0.5–4 h), and 13 (4 h) of the MOR-1 clone (Fig. 6, A, C, and D). In contrast, short-term deprivation-induced intake was significantly and consistently increased by AS ODN probes directed against coding exons 3 (0.5–4 h), 5a (0.5–1 h), and 9 (0.5–1, 4 h) of the MOR-1 clone (Fig. 6, A–C). Moreover, a significant biphasic effect upon short-term deprivation-induced intake was observed by AS ODN probes directed against coding exons 1 (0.5–1 h, increase; 4 h, decrease) and 10 (0.5 h, increase; 2–4 h, decrease) of the MOR-1 clone (Fig. 6, A and D). Finally, short-term deprivation-induced intake failed to be affected by AS ODN probes directed against coding exons 6 and 12 of the MOR-1 clone (Fig. 6, B and D).

Significant differences in longer-term (24–48 h) intake were observed among control and MOR-1 AS ODN conditions [$F(12,1032) = 179.19$, $P < 0.0001$], across test times [$F(1,86) = 3051.82$, $P < 0.0001$], and for the interaction between conditions and times [$F(12,1032) = 72.11$, $P < 0.0001$]. Longer-term deprivation-induced intake was significantly reduced by an AS ODN probe directed against coding exon 4 (24 h) of the MOR-1 clone (Fig. 7A). In contrast, longer-term deprivation-induced intake was significantly and consistently increased by AS ODN probes directed against coding exons 3 (24–48 h), 5a (24–48 h), 6 (24–48 h), 9 (48 h), 12 (24–48 h), and 13 (24–48 h) of the MOR-1 clone (Fig. 7, A-D). Finally, longer-term deprivation-induced in-

TABLE 3
Body weight loss and recovery in 12-h food-deprived mice treated with general and selective opioid receptor antagonists

Treatment	Body Weight Loss Mean (±S.E.M.)	Body Weight Recovery Mean (±S.E.M.)	
		24 h Post	48 h Post
	g		
Control	−4.38 (0.21)	4.06 (0.21)	4.39 (0.26)
Naltrexone, 5.0 mg/kg s.c.	−4.41 (0.28)	3.27 (0.35)*	4.94 (0.40)
Naltrexone, 50 μg i.c.v.	−3.86 (0.34)	3.62 (0.58)	3.77 (0.41)
βFNA, 20 μg	−3.63 (0.44)	3.35 (0.46)*	3.68 (0.76)*
NBNI, 20 μg	−3.85 (0.40)	2.17 (0.58)*	3.66 (0.48)*
NTI, 20 μg	−4.01 (0.40)	3.61 (0.96)	5.57 (1.02)*

*Significant difference relative to corresponding control value.

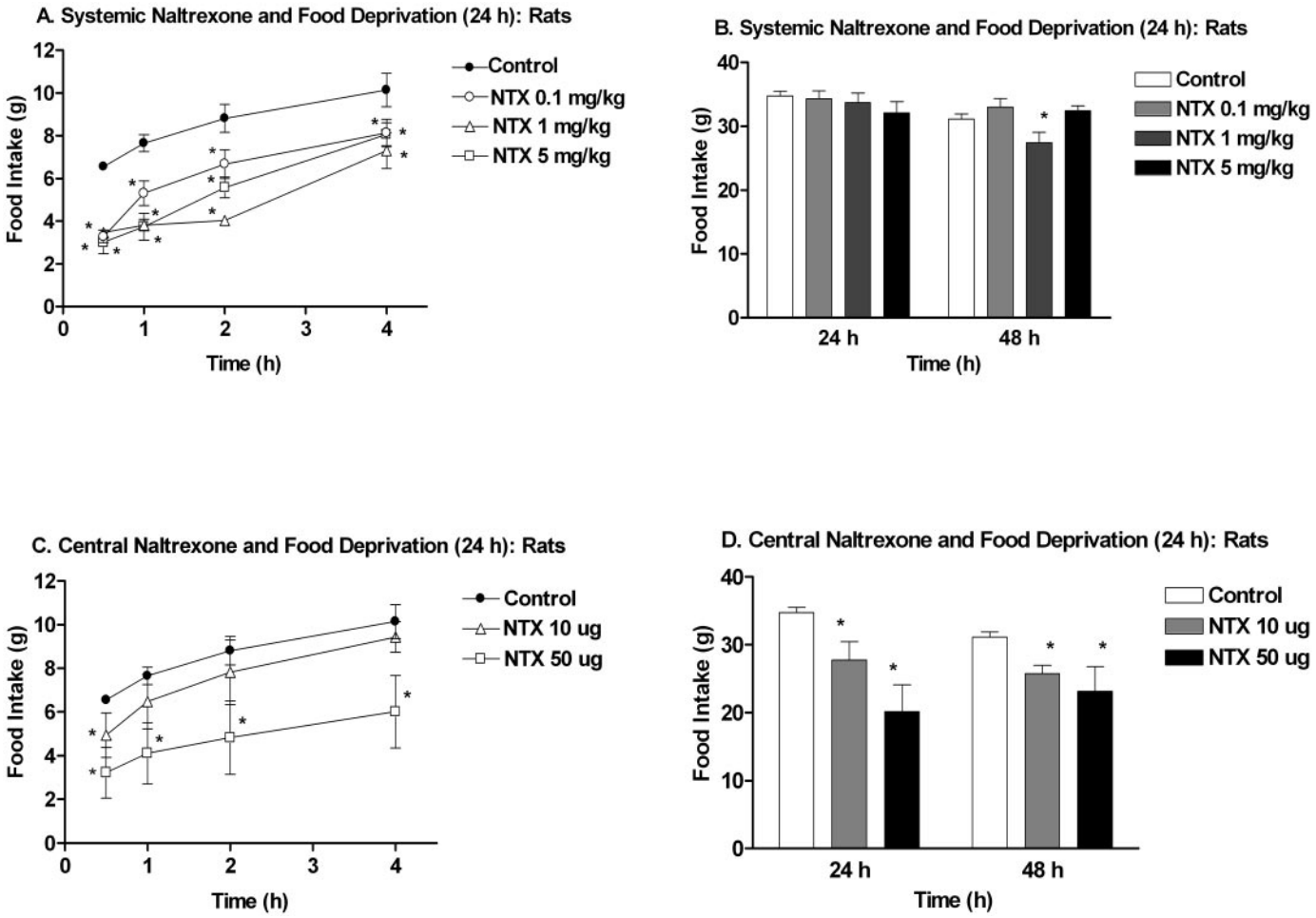

Fig. 4. Alterations in short-term (0.5–4 h; panels A and C) and longer-term (24–48 h; panels B and D) cumulative intake following systemic (panels A and B) or ventricular (panels C and D) administration of naltrexone in rats previously deprived of food for 24 h. The asterisks denote significant differences (Tukey's comparisons, $P < 0.05$) from corresponding vehicle control conditions.

take failed to be affected by AS ODN probes directed against coding exons 1, 2, 7, 8, and 10 of the MOR-1 clone (Fig. 7, A, B, and D).

DOP, KOP, and NOP AS Effects on Intake. Significant differences in short-term (0.5–4 h) intake were observed among control and DOP, KOP, and NOP AS ODN conditions [$F(9,774) = 190.47$, $P < 0.0001$], across test times [$F(3,258) = 7174.17$, $P < 0.0001$], and for the interaction between conditions and times [$F(27,2322) = 63.28$, $P < 0.0001$]. Short-term deprivation-induced intake was significantly and consistently reduced by AS ODN probes directed against coding exons 1 (1–4 h), 2 (1–4 h), and 3 (1–4 h) of the DOP gene (Fig. 8A), coding exons 1 (2–4 h), 2 (2–4 h), and 3 (0.5–4 h) of the KOP gene (Fig. 8C), and coding exon 1 (2–4 h) of the NOP gene (Fig. 8E). In contrast, short-term deprivation-induced intake was significantly and consistently increased by an AS ODN probe directed against coding exon 2 (0.5–4 h) of the NOP gene (Fig. 8E).

Significant differences in longer-term (24–48 h) intake were observed among control and DOP, KOP, and NOP AS ODN conditions [$F(9,774) = 44.84$, $P < 0.0001$], across test times [$F(1,86) = 3168.20$, $P < 0.0001$], and for the interaction between conditions and times [$F(9,774) = 68.36$, $P < 0.0001$]. Longer-term deprivation-induced intake was significantly increased by AS ODN probes directed against coding exon 3 (48 h) of the KOP gene (Fig. 8D) and coding exon 1 (48 h) of the NOP gene (Fig. 8F). Yet, longer-term deprivation-induced intake failed to be affected by AS ODN probes directed against coding exons 1, 2, and 3 of the DOP gene (Fig. 8B), coding exons 1 and 2 of the KOP gene (Fig. 8D), and coding exon 2 of the NOP gene (Fig. 8F).

Opioid AS Effects on Weight. Significant differences in deprivation-induced body weight loss occurred among control and MOR-1 AS ODN conditions [$F(12,211) = 2.08$, $P < 0.019$], but not among control and DOP, KOP, or NOP conditions [$F(8,182) = 1.45$, n.s.]. Significant changes in weight loss were greater following AS ODN probes directed against coding exons 9 and 10 of the MOR-1 gene and were significantly less following an AS ODN probe directed against coding exon 2 of the MOR-1 gene (Table 5). Significant differences in body weight recovery following deprivation were observed among control and MOR-1 AS ODN conditions [$F(12,1524) = 212.68$, $P < 0.0001$], across test times [$F(1,127) = 1604.21$, $P < 0.0001$], and for the interaction between conditions and times [$F(12,1524) = 32.31$, $P < 0.0001$]. Significant differences in body weight recovery following deprivation were observed among control and DOP, KOP, and NOP AS ODN conditions [$F(8,1016) = 160.53$, $P < 0.0001$], across test times [$F(1,127) = 760.09$, $P < 0.0001$], and for the interaction between conditions and times

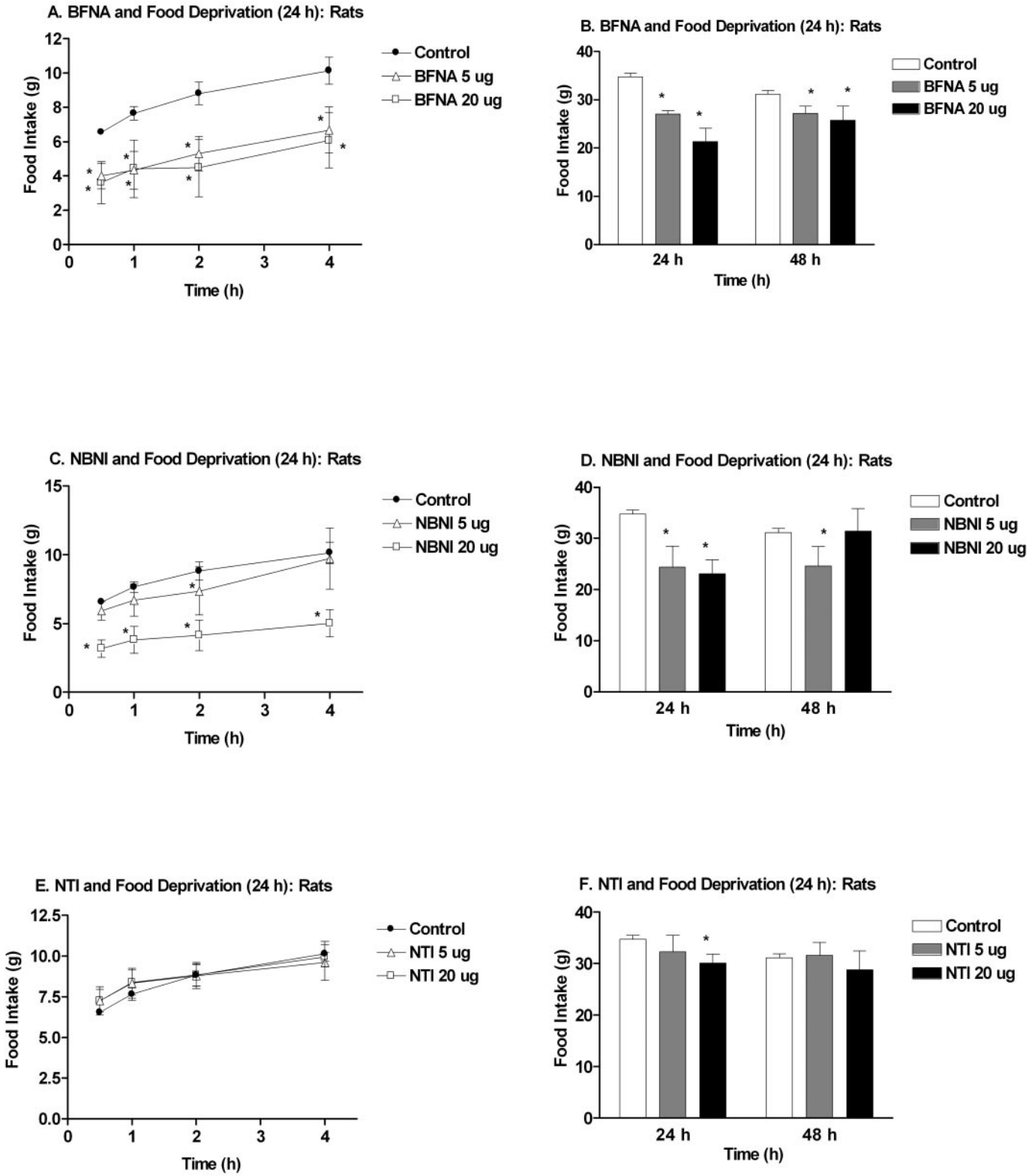

Fig. 5. Alterations in short-term (0.5–4 h; panels A, C, and E) and longer-term (24–48 h; panels B, D, and F) cumulative intake following ventricular administration of selective μ (βFNA; panels A and B), κ (NBNI; panels C and D), and δ (NTI; panels E and F) opioid receptor subtype antagonists in rats previously deprived of food for 24 h. The asterisks denote significant differences (Tukey's comparisons, $P < 0.05$) from corresponding vehicle control conditions.

[$F(8,1016) = 96.05$, $P < 0.0001$]. Deprivation-induced weight recovery was significantly and consistently *reduced* by AS ODN probes directed against coding exons 1 (24–48 h), 4 (24–48 h), 5a (24 h), and 7 (24–48 h) of the MOR-1 clone (Table 5), coding exons 1 (24–48 h) and 2 (48 h) of the DOP gene (Table 6), coding exon 1 (48 h) of the KOP gene (Table 6),

TABLE 4
Body weight loss and recovery in 24-h food-deprived rats treated with general and selective opioid receptor antagonists

Treatment	Body Weight Loss Mean (± S.E.M.)	Body Weight Recovery Mean (±S.E.M.)	
		24 h Post	48 h Post
	g		
Control	−40.70 (1.00)	31.60 (1.45)	40.70 (1.75)
Systemic naltrexone, s.c.			
0.1 mg/kg	−41.17 (1.68)	26.17 (1.85)*	32.50 (2.16)*
1.0 mg/kg	−35.60 (2.73)	23.40 (4.00)*	26.20 (0.97)*
5.0 mg/kg	−36.40 (1.50)	26.60 (2.73)*	24.60 (2.74)*
Central naltrexone, i.c.v.			
10 μg	−39.67 (3.00)	12.50 (4.60)*	17.33 (4.19)*
50 μg	−42.20 (4.65)	4.60 (9.20)*	6.00 (9.35)*
βFNA			
5 μg	−38.80 (2.15)	25.60 (0.93)*	25.20 (2.31)*
20 μg	−46.00 (1.32)	16.60 (6.93)*	21.80 (6.10)*
NBNI			
5 μg	−36.00 (4.37)	6.40 (6.95)*	13.20 (6.71)*
20 μg	−39.60 (1.43)	11.60 (2.69)*	17.80 (1.32)*
NTI			
5 μg	−49.60 (2.11)*	20.00 (5.09)*	28.20 (6.95)*
20 μg	−43.40 (2.17)	13.00 (2.45)*	22.20 (2.08)*

*Significant difference relative to corresponding control value.

and coding exons 1 (24–48 h) and 2 (24–48 h) of the NOP gene (Table 6). In contrast, deprivation-induced weight recovery was significantly and consistently *increased* by AS ODN probes directed against coding exons 6 (48 h) and 9 (24–48 h) of the MOR-1 clone (Table 5). Yet, deprivation-induced weight recovery *failed* to be affected by AS ODN probes directed against coding exons 2, 3, 7, 8, 10, 12, and 13 of the MOR-1 clone, coding exon 3 of the DOP gene, and coding exons 2 and 3 of the KOP gene (Tables 5 and 6).

Discussion

The present study combined pharmacological antagonist and antisense approaches to provide converging evidence for the relative contributions of different opioid receptors and genes in the regulation of the homeostatic challenge of food deprivation. Feeding behavior has traditionally been studied in rats, mostly because of their similar ingestive responses to other species and the measurable magnitude of their consumatory behavior. Previous studies with general (Holtzman, 1974; Brown and Holtzman, 1979; Frenk and Rogers, 1979; Cooper, 1980) and selective (Simone et al., 1985; Arjune et al., 1990, 1991; Levine et al., 1990, 1991; Koch and Bodnar, 1994) opioid receptor subtype antagonists revealed that deprivation-induced feeding in the rat is potently reduced by general and μ, moderately reduced by κ, and minimally affected by δ receptor antagonists. The effects of antisense probes directed against the DOP and KOP genes upon deprivation-induced feeding paralleled the antagonist effects in rats, yet antisense probes directed against exons of the MOP gene produced, at best, modest effects (Hadjimarkou et al., 2003b). The explanation that additional isoforms of the MOR-1 clone (Bare et al., 1994; Zimprich et al., 1995; Pan et al., 1999, 2000, 2001; Pasternak and Pan, 2000; Pasternak, 2001) mediated deprivation-induced feeding was the central rationale for conducting this study. Because the mouse is the species for which these extended exons were identified and because selective opioid antagonist effects upon deprivation-induced feeding have not been evaluated in mice, comparisons needed to be made in this species. The following sections detail the findings and implications.

Species Comparisons of General Opioid Antagonism and Deprivation-Induced Feeding. As expected, both systemic and ventricular administration of the general opioid antagonist naltrexone dose dependently (0.1–5 mg/kg) and potently (24–54%) reduced deprivation- induced intake in the rat (24–54%) and the mouse (40–78%). Peak antagonist effects were typically observed at 0.5 to 1 h following administration, and the magnitude (20%) of reductions declined 24 and 48 h following food reintroduction in both species. Moreover, an almost identical pattern of naltrexone effects was observed in mice deprived for only 12 h during the dark cycle with reductions noted following systemic (20–39%) and central (70–82%) administration.

Species Comparisons of Selective Opioid Antagonism and Deprivation-Induced Feeding. Strong short-term (0.5–4 h) reductions in deprivation (24 h)-induced intake following the μ-selective opioid antagonist βFNA in rats (35–50%) and mice (46–50%) were consistent with previous studies in rats (Arjune et al., 1990; Levine et al., 1991; Koch and Bodnar, 1994). Longer-term (24–48 h) μ opioid antagonist effects were also similar for both mice and rats deprived of food for 24 h. Although μ opioid antagonism more modestly reduced intake (~20%) following the shorter (12 h) deprivation period in the mouse, it appears that μ opioid antagonism exerts quite comparable inhibitory actions upon deprivation-induced intake in both species.

Similar conclusions can be drawn for the κ-mediated effects. Thus, NBNI dose dependently and comparably reduced short-term deprivation-induced intake in rats (16–53%) and mice (12 and 24 h, 20–53%), consistent with the 30% reductions observed previously (Levine et al., 1990; Koch and Bodnar, 1994). Longer-term (24–48 h) intakes were significantly reduced by NBNI in the rat (20–34%), but to a lesser degree in the mouse (8–29%).

A species-specific pattern emerged for δ-mediated effects upon deprivation-induced intake. Consistent with previous observations (Arjune et al., 1991; Koch and Bodnar, 1994),

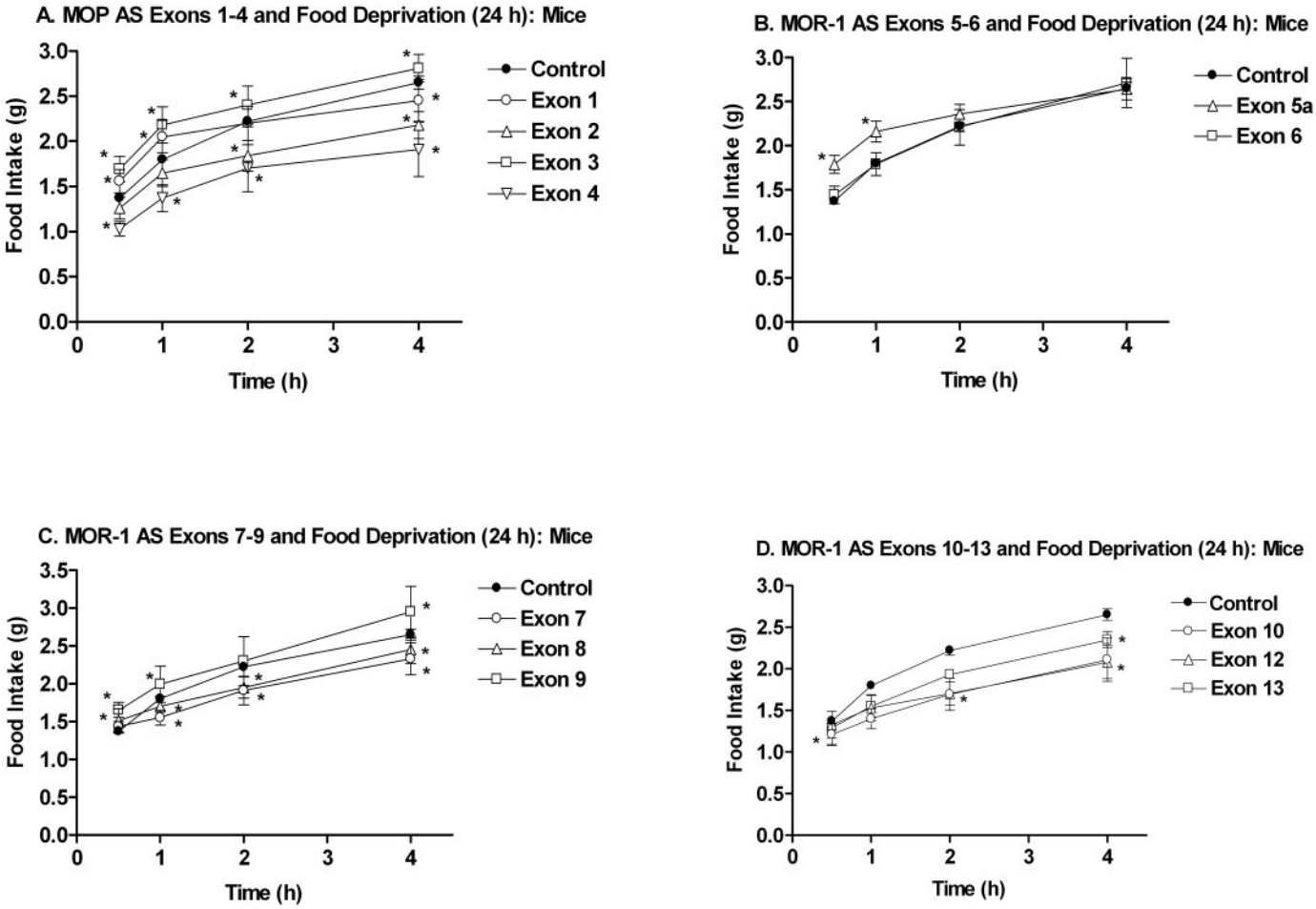

Fig. 6. Alterations in short-term (0.5–4 h) cumulative intake following ventricular administration of antisense probes directed against the four exons of the MOP gene (exons 1, 2, 3, and 4; panel A), two extended MOR-1 exons (exons 5a and 6; panel B), three extended exons of the MOR-1C clone (exons 7, 8, and 9; panel C), and three other extended MOR-1 exons (exons 10, 12, and 13; panel D) in mice previously deprived of food for 24 h. The asterisks denote significant differences (Tukey's comparisons, $P < 0.05$) from corresponding vehicle control conditions.

the δ opioid antagonist NTI failed to appreciably alter deprivation-induced feeding in rats, yet an identical dose range significantly reduced short-term deprivation-induced intake in the mouse after 24 (28–39%) and 12 (~20%) h. These δ-mediated effects dissipated in the mouse after 24 to 48 h (13–15%). Thus, these data suggest a species-specific role for δ opioid receptors in mediating deprivation-induced intake in mice but not rats.

Species Comparisons of NOP Antisense Probes and Deprivation-Induced Feeding. Antisense probes directed against exons of the NOP gene potently reduce feeding elicited by either orphanin FQ/nociceptin (exons 1, 2, and 3; Leventhal et al., 1998a) or dynorphin (exons 1 and 2; Silva et al., 2002), while exerting relatively minor effects upon intake elicited by M6G, β-endorphin, 2DG, or mercaptoacetate (Burdick et al., 1998; Leventhal et al., 1998b; Stein et al., 2000; Silva et al., 2001). A similar modest role for the NOP gene can be observed for deprivation-induced feeding in both species. An antisense probe directed against exon 1, but not exon 2, of the NOP gene modestly reduced deprivation-induced feeding in the mouse, similar to that observed in the rat (Hadjimarkou et al., 2003b).

Species Comparisons of KOP Antisense Probes and Deprivation-Induced Feeding. Antisense probes directed against exons of the KOP gene effectively reduce feeding elicited by dynorphin and β-endorphin (Silva et al., 2001, 2002) as well as by glucoprivic and lipoprivic challenges (Burdick et al., 1998; Stein et al., 2000). Deprivation-induced feeding in the rat was potently (43%) reduced by an antisense probe directed against exon 2 of the KOP gene (Hadjimarkou et al., 2003b). Similar patterns were noted in the mouse, in that antisense probes directed against exons 1 and 2, but especially exon 3 (26–35%) of the KOP gene, significantly reduced deprivation-induced feeding. Thus, KOP antisense and NBNI actions upon deprivation-induced feeding in both mice and rats provide strong and converging evidence for κ-mediated actions in this homeostatic challenge in both species.

Species Comparisons of DOP Antisense Probes and Deprivation-Induced Feeding. Antisense probes directed against exons of the DOP gene potently reduce feeding elicited by the δ_2 agonist, deltorphin (Leventhal et al., 1998), but more modestly reduce intake elicited by dynorphin, β-endorphin, 2DG, and mercaptoacetate (Burdick et al., 1998; Stein et al., 2000; Silva et al., 2001, 2002). The relative ineffectiveness of δ antagonism to reduce deprivation-induced intake in rats (Arjune et al., 1991; Koch and Bodnar, 1994; Ragnauth et al., 1997) is consistent with the very modest reductions in deprivation-induced feeding following antisense probes directed against exon 1 of the DOP gene (Hadjimarkou et al.,

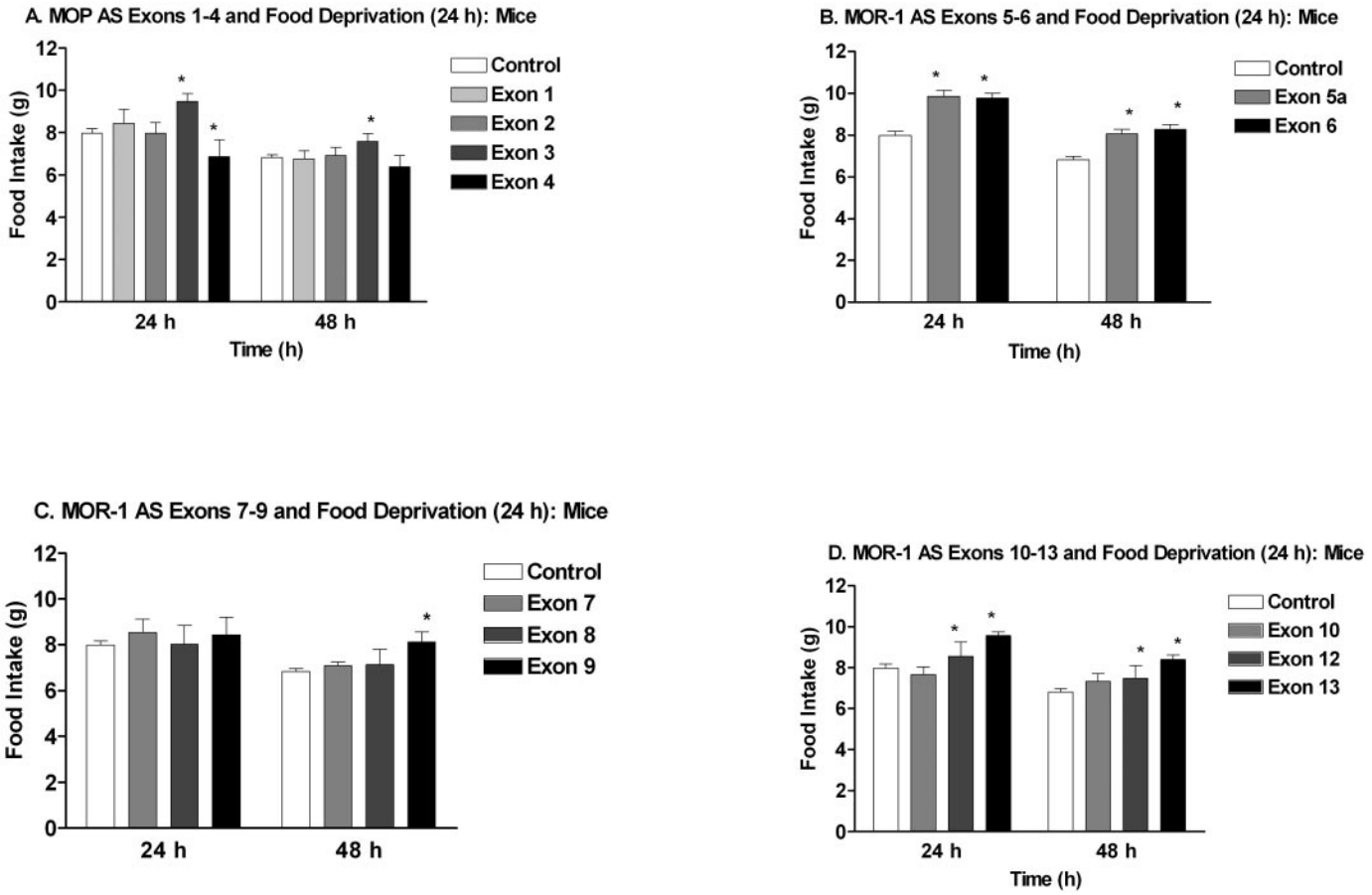

Fig. 7. Alterations in longer-term (24–48 h) cumulative intake following ventricular administration of antisense probes directed against the four exons of the MOP gene (exons 1, 2, 3, and 4; panel A), two extended MOR-1 exons (exons 5a and 6; panel B), three extended exons of the MOR-1C clone (exons 7, 8, and 9; panel C), and three other extended MOR-1 exons (exons 10, 12, and 13; panel D) in mice previously deprived of food for 24 h. The asterisks denote significant differences (Tukey's comparisons, $P < 0.05$) from corresponding vehicle control conditions.

2003b). The species-specific actions of δ antagonist-induced reductions in deprivation-induced intake in the mouse were confirmed because short-term deprivation-induced intake was significantly reduced by antisense probes directed against all three exons (11–24%) of the DOP gene in the mouse. Thus, convergence between the δ opioid antagonist and DOP antisense approaches not only confirmed the existence of δ-mediated effects in the mouse, but also indicated their absence in the rat.

MOP Antisense Probes and Deprivation-Induced Feeding in the Mouse. Antisense probes directed against the MOP gene have typically produced the most pronounced reductions in intake regulated by either homeostatic responses, spontaneous intake and body weight (Leventhal et al., 1996), 2DG-induced glucoprivation (Burdick et al., 1998), mercaptoacetate-induced lipoprivation (Stein et al., 2000), or opioid agonists (Leventhal et al., 1997, 1998; Silva et al., 2001, 2002). Indeed, antisense probes directed against exons of the MOP gene differentiated between μ-mediated actions of feeding responses elicited by morphine and DAMGO (exons 1 and 4) relative to the morphine metabolite, M6G (exons 2 and 3) (Leventhal et al., 1997, 1998). These data, together with similar effects on analgesic responses, suggested a functional significance of MOR-1 isoforms (e.g., Bare et al., 1994; Zimprich et al., 1995; Pan et al., 1999, 2000, 2001; Pasternak and Pan, 2000; Pasternak, 2001). In these isoforms, exons 1, 2, and 3 are conserved for the most part, and thus the last exon(s) differentiate them from each other.

Traditional MOP antisense probes (exons 2, 3, and 4; ~30%) that produced modest effects in the rat (Hadjimarkou et al., 2003b) also did so in the mouse: exon 1 AS (18%), 2 AS (18%), or 4 AS (23–28%). Immunohistochemical studies using antibodies raised against different MOR-1 exons demonstrate differential site-specific distributions for the MOP gene and its splice variants (Abbadie et al., 2000a,b, 2001; Abbadie and Pasternak, 2001). Interestingly, the distribution of immunoreactivity for the MOP gene and MOR-1C splice variant can be found in nuclei relevant to the opioid control of feeding behavior, including the paraventricular, arcuate, and ventromedial nuclei of the hypothalamus, lateral septum, nucleus accumbens, amygdala, parabrachial nucleus, and nucleus of the solitary tract. These nuclei support feeding responses induced by opioid agonists (see review, Bodnar, 2004), and moreover, food restriction or food deprivation alter opioid peptide and receptor gene expression or protein levels in a number of these sites (e.g., Berman et al., 1994; Wolinsky et al., 1994, 1996; Kim et al., 1996). Interestingly, whereas neither food deprivation nor food restriction altered immunoreactivity of the MOP gene in any of these sites, food restriction selectively increased MOR-1C immunoreactivity in the dorsal and ventral parvocellular subdivisions of the paraventricular nucleus, whereas both food restriction and

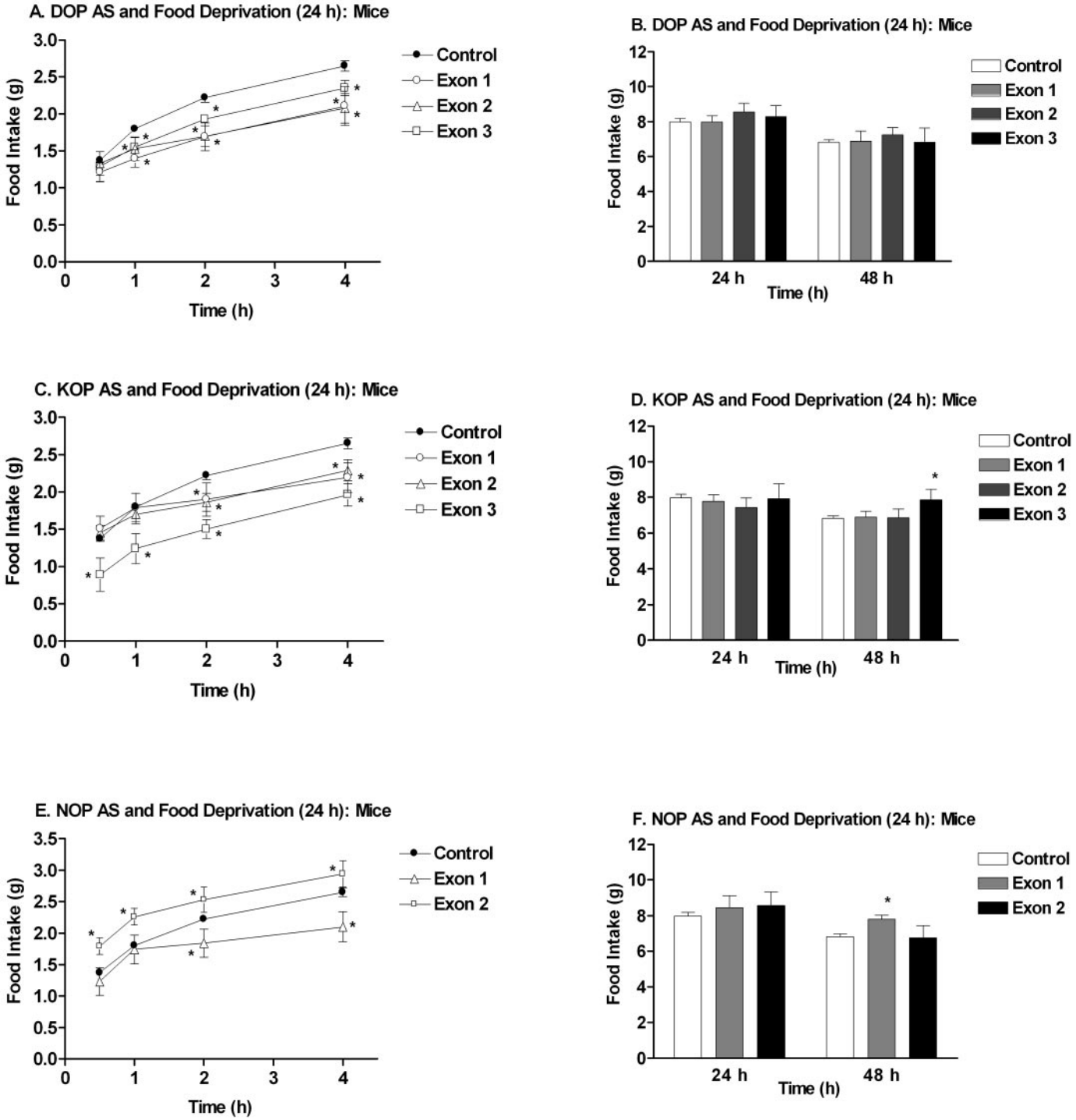

Fig. 8. Alterations in short-term (0.5–4 h; panels A, C, and E) and longer-term (24–48 h; panels B, D, and F) cumulative intake following ventricular administration of antisense probes directed against the three exons of the DOP gene (panels A and B), the three exons of the KOP gene (panels C and D), and two exons of the NOP gene (panels E and F) in mice previously deprived of food for 24 h. The asterisks denote significant differences (Tukey's comparisons, $P < 0.05$) from corresponding vehicle control conditions.

food deprivation decreased MOR-1C immunoreactivity in the nucleus of the solitary tract (Hadjimarkou et al., 2003a). Yet, modest but significant reductions in deprivation-induced intake were noted in the mouse by antisense probes directed against exons 7 (14%) or 8 (12%), and the antisense probe directed against exon 9 actually increased deprivation-induced feeding (10–17%). None of the other extended antisense probes produced any further marked effects. Hence, one cannot clearly correlate pronounced μ antagonist effects with weaker individual antisense effects, suggesting mediation by multiple MOP isoforms.

Magnitudes of Antagonist and Antisense Probe Effects upon Deprivation-Induced Feeding. Previous studies comparing antagonist and AS ODN probe effects upon feeding elicited by the opioid agonists, morphine (Leventhal et al., 1998b), DAMGO (Leventhal et al., 1997), M6G (Lev-

TABLE 5
Body weight loss and recovery in 24-h food-deprived mice treated with MOP antisense probes targeted against exons 1, 2, 3, 4, 5a, 6, 7, 8, 9, 10, 12, and 13

Treatment	Body Weight Loss Mean (±S.E.M.)	Body Weight Recovery Mean (±S.E.M.)	
		24 h Post	48 h Post
	g		
Control	−5.04 (0.11)	4.84 (0.15)	5.28 (0.17)
MOP gene			
Exon 1	−4.58 (0.32)	3.64 (0.39)*	3.64 (0.48)*
Exon 2	−3.94 (0.83)*	5.21 (0.39)	5.41 (0.65)
Exon 3	−4.90 (0.30)	4.45 (0.19)	5.00 (0.28)
Exon 4	−5.55 (0.50)	4.03 (0.83)*	4.65 (1.08)*
Exon 5a	−4.41 (0.43)	4.21 (0.26)*	5.18 (1.70)
Exon 6	−4.11 (0.59)	5.84 (0.64)*	6.78 (0.87)*
Exon 7	−4.40 (1.13)	3.98 (1.13)*	4.51 (1.10)*
Exon 8	−5.16 (0.36)	4.94 (0.41)	5.53 (0.77)
Exon 9	−6.08 (0.54)*	6.09 (0.42)*	6.78 (0.41)*
Exon 10	−6.23 (0.59)*	5.35 (0.54)	5.70 (0.94)
Exon 12	−5.56 (0.25)	4.69 (0.68)	4.94 (0.98)
Exon 13	−4.54 (0.64)	4.66 (0.32)	5.40 (0.43)

*Significant reduction relative to corresponding control value.

TABLE 6
Body weight loss and recovery in 24-h food-deprived mice treated with antisense probes targeted against the coding exons of the DOP (exons 1–3), KOP (exons 1–3), and NOP (exons 1–2) genes

Treatment	Body Weight Loss Mean (±S.E.M.)	Body Weight Recovery Mean (±S.E.M.)	
		24 h Post	48 h Post
	g		
Control	−5.04 (0.11)	4.84 (0.15)	5.28 (0.17)
DOP gene			
Exon 1	−3.83 (1.12)	3.88 (0.59)*	4.25 (0.89)*
Exon 2	−4.94 (0.22)	4.31 (0.35)	4.54 (0.49)*
Exon 3	−4.91 (0.46)	4.98 (0.50)	4.69 (0.74)
KOP gene			
Exon 1	−5.11 (0.19)	4.46 (0.28)	4.55 (0.85)*
Exon 2	−5.63 (0.21)	4.51 (0.47)	5.94 (1.27)
Exon 3	−4.26 (0.64)	4.28 (0.96)	5.76 (0.61)
NOP gene			
Exon 1	−4.78 (0.28)	3.29 (0.51)*	3.93 (0.38)*
Exon 2	−4.44 (0.43)	3.23 (0.64)*	2.87 (1.19)*

*Significant reduction relative to corresponding control value.

enthal et al., 1998b), β-endorphin (Silva et al., 2001), and dynorphin A (Silva et al., 2002) have demonstrated that equipotent reductions are observed when the appropriate antagonist and AS ODN probes are used. Such a pattern of comparable antagonist and AS ODN probe effects has also been observed for feeding responses elicited by 2DG-induced glucoprivation (Arjune and Bodnar, 1990; Arjune et al., 1990, 1991; Burdick et al., 1998) and mercaptoacetate-induced lipoprivation (Stein et al., 2000). As indicated through the discussion, the magnitude of the AS ODN effects were typically more modest than corresponding antagonist effects for deprivation-induced feeding for mice in the present study and for rats in previous work (Hadjimarkou et al., 2003b). The major difference between the AS ODN probes and antagonists is selectivity. Although the antagonist will essentially block the entire receptor under study, the AS ODN probe directed against a given exon of the receptor gene will only block that exon (or that variant) at a given time. Therefore, the ability to observe a given size effect may require different levels of blockade. It may involve the level of blockade at each subtype of the receptor or alternatively the ability to block multiple subtypes of the receptor. This latter point is well illustrated by the ability of the μ antagonist, βFNA, to block M6G- or DAMGO-induced feeding, but the differential ability of MOP AS ODN probes to block feeding induced by M6G (exons 2 and 3) and DAMGO (exons 1 and 4) (Leventhal et al., 1997, 1998b). Furthermore, whereas antagonists at sufficiently high doses can block all of the receptors, AS ODN probes typically reduce receptor number by only 50%. In the agonist, glucoprivic, and lipoprivic feeding paradigms, this AS ODN probe-induced reduction may be sufficient to produce the equipotent effect to that of the antagonist. It appears that the feeding response elicited by food deprivation is more powerfully driven, and hence would need greater blockade of the relevant receptors. In this regard, it should be noted that even the magnitude of the antagonist-induced reductions upon deprivation-induced feeding (~30–70%) is less than that observed for feeding elicited by opioid agonists or other homeostatic challenges (~80–100%) (see review, Bodnar, 2004). This latter point is consistent with the concept that food deprivation, as the ultimate homeostatic challenge, would necessarily activate many relevant neurotransmitter and neuropeptide receptor systems, and hence need the full blockade of those receptors to interfere fully with the expression of the feeding response.

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Address correspondence to: Dr. Richard J. Bodnar, Department of Psychology, Queens College, CUNY, 65-30 Kissena Blvd., Flushing, NY 11367. E-mail: richard_bodnar@qc.edu