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Effects of butorphanol on feeding and neuropeptide Y in the rat

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ABSTRACT

Butorphanol ([BT] an opioid receptor agonist/antagonist) is different from other opioid agonists in that a single dose of BT can elicit up to 12 g of chow intake in a satiated rat whereas most opioid agonists induce a mild feeding response (2–3 g). Here, we first examined whether the effectiveness of BT to elicit feeding was affected by dose, method of infusion and possible tachyphylaxis following administration. Secondly, we examined whether BT administration influenced hypothalamic NPY gene expression and peptide levels. A single dose administration of BT (4 mg/kg) significantly increased food intake at 2, 3 and 6 h after administration. However following repeated injections of BT at 4 mg/kg, the cumulative long-term intake of BT-treated rats did not differ from that of controls, indicating that the animals compensate for the increased feeding following BT injection by decreased feeding at a later time. An ascending dose schedule of repeated BT injections resulted in additional feeding. NPY gene expression in the ARC was influenced by how much food had been consumed, but not by BT. The amount of food consumed and the level of NPY mRNA were inversely correlated. This is consistent with NPY's role in normal feeding. BT treatment did not affect either NPY or leptin RIA levels. We conclude that the feeding produced by BT is sensitive to dose and dosing paradigm. Further, its mechanism of action does not appear to be mediated by NPY or leptin pathways.

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1. Introduction

The role of opioids in feeding is well established: administration of opioid agonists increase feeding, while opioid receptor antagonist administration decreases feeding; for review see Bodnar (2004), and Gosnell and Levine (2009). Furthermore, feeding status (i.e. deprived vs. satiated) influences mRNA levels of opioid peptides in the brain (Kim et al., 1996).

There are several opioid receptor subtypes with differential distribution both centrally and peripherally. The subtypes with the most well defined roles in feeding behavior are the mu, kappa and delta opioid receptors. Stimulation of the mu receptor induces the most potent feeding response, whereas stimulation of the kappa and delta opioid receptors results in more modest feeding.

Butorphanol tartrate (BT) is a synthetic opioid agonist/antagonist; BT's receptor profile remains unclear, but the literature suggests that it is primarily a kappa and mu-receptor agonist, but has some affinity for the delta-receptor (Boggiano et al., 2005). However based on its reported affinity ratio of 1:4:25 for mu, delta and kappa receptors, respectively (Chang et al., 1981; Lahti et al., 1985), it would seem that BT has a greater affinity for delta-receptors as compared with mu-receptors. The testing conditions have been found to be important in eliciting butorphanol's actions as either a kappa/mu agonist, a selective mu agonist or even a mu antagonist (Walsh et al., 2008). BT has very potent effects on feeding (Levine et al., 1994; Levine and Morley, 1983; Morley et al., 1985). In Sprague–Dawley rats, at doses ranging from 2 to 16 mg/kg of BT, 7–11 g of chow was consumed in the first 6 h, compared with only 3 g of chow in control rats (Levine et al., 1994). A possible explanation for its potent effect on feeding is that the simultaneous stimulation of both mu- and kappa opioid receptor subtypes by BT allows for an additive feeding effect. The orexigenic effect of BT is blocked by peripheral naloxone administration (Levine et al., 1994). As peripherally administered naloxone crosses the blood brain barrier, and distributes throughout the brain, this suggests that the feeding effects of BT may be mediated through central opioid pathways. However peripheral injections of BT seem to more robustly increase feeding as compared with intracerebroventricular administration (Levine et al., 1994)or site-specific (PVN and CeA; Kim et al., 2001) injections of BT. It is feasible that peripheral administration of BT allows for activation of a wider brain network of opioid sensitive sites, as compared with the central administration. Presently the mechanism by which BT influences feeding is unknown. One possibility is that opioid metabolites, such as norbutorphanol and hydroxybutorphanol which are by-products of peripheral metabolism (Niwa et al., 1985) are responsible for the feeding effects of peripherally administered butorphanol.

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Another possibility is that butorphanol induced feeding results either by the stimulation of central feeding pathways or the blockade of a tonic feeding-inhibitory pathway. There are also several lines of evidence suggesting that opioids interact with neuropeptide Y (NPY). NPY is a member of the pancreatic polypeptide family and is the most potent stimulator of feeding known. Central administration of NPY results in robust increases in feeding. Central (ICV, NTS, and CeA) and peripheral administration of specific opioid antagonists block feeding produced by centrally administered NPY (Levine et al., 2004a). Peripheral naltrexone administration results in up-regulation of hypothalamic NPY (Kotz et al., 1996), while peripheral naloxone administration resulted in increased NPY concentration in the dorsomedial hypothalamic nucleus (Lambert et al., 1994). These data point to an interaction between endogenous NPY and opioids.

Finally, leptin is an important afferent signal of caloric status, and is involved in food intake regulation. It is important to consider that neural circuits that regulate metabolism overlap with those that regulate reward. Exogenous leptin administration has been shown to not only reduce food intake and body weight, but has also resulted in reduced responding for palatable foods (Figlewicz et al., 2006) and has also been shown to reduce the strength of conditioned place preference for a high-fat diet (Figlewicz, 2003).

The present studies were undertaken to explore the feeding effects of peripherally administered BT, and to determine whether hypothalamic NPY is altered in response to butorphanol administration. First we set out to determine the factors that may influence the potency of butorphanol feeding effects: dose, duration and method of infusion, and possible tachyphylaxis following administration. Secondly we sought to determine whether BT administration influenced hypothalamic NPY gene expression and peptide levels and circulating leptin levels. The final experiment in the present series was conducted to examine whether BT administration which stimulates food intake, was able to counteract the effects of 24 h food deprivation, thereby maintaining a higher circulating leptin level in the BT treated rats. We hypothesized that BT administration would result in reduced hypothalamic arcuate NPY gene expression and it would result in higher levels of circulating leptin.

2. Methods

2.1. Animals

Male Sprague Dawley rats (Harlan, Madison, WI) weighing 272 ± 2 g were individually housed in standard hanging cages with a 12 h light/12 h dark photoperiod (lights on at 7 am, lights off at 7 pm) in temperature controlled rooms (21–22 °C). Body weight at the time of the experiments was 302 ± 2 g. Teklad certified rodent chow and water were allowed ad libitum except where noted.

2.2. Drugs

Butorphanol tartrate (Stadol®) was purchased from Bristol-Myers Squibb Co. (Princeton, NJ). Appropriate concentrations were made by dilution with saline (0.9%).

2.3. Injections

Butorphanol injections were given subcutaneously (s.c.) in a 0.5 ml volume near the hind leg. In studies with several repeated injections, the injection site was rotated from one leg to the other in order to avoid irritation at the injection site.

2.4. Food intake measurements

Except where noted, the rats were given ad libitum access to the rodent chow. For all food intake tests, rats were returned to their home cages following injections. Immediately prior to an injection, the chow was removed from their cages. Immediately post-injection, pre-weighed pellets of chow were placed inside the rats' food hoppers. The pellets and the resulting spillage were weighed at specific time points post-injection in order to quantify the rats' intake. At each point, the weight of the hopper and the remaining food and spillage were subtracted from the original weight to determine the amount consumed.

2.5. Osmotic pump implantation

In Experiment III, osmotic minipumps (Alzet, model 2ML1, 10 μl/h, Alza Corp., Palo Alto, CA) pre-filled with either 0.9% saline or butorphanol tartrate (0.0254 mg/μl) were pre-incubated overnight at 37 °C to allow the pumps to reach maximum pumping rate by the time of implantation. These pumps were then implanted s.c. by making a small incision on the right side (midway between dorsal–ventral planes) of the rats, approximately halfway between the front and back legs. The rats were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL), a short acting inhalant anesthetic. The amount of butorphanol in the pump was calculated to deliver the same total amount of butorphanol (36 mg/kg) as that of 4 mg/kg injected every 6 h for 48 h. Implantation of the pumps took less than 1 min per rat, and the length of the anesthesia was approximately 5 min per rat. At the end of the study, the pumps were removed and checked for fluid depletion to verify correct functioning.

2.6. Euthanization and tissue harvesting

After the experimental period, rats were sacrificed by rapid decapitation. The brains were rapidly excised, chilled in ice-cold saline and sliced using a Stoelting tissue slicer and were sectioned with cuts at 0, +2 and +5.5 mm relative to the anterior commissure, corresponding to the brain atlas of Paxinos and Watson (1986). A single 3 mm punch of PVN was taken from the second slice (0 to +2 mm); a scalpel cut was used to remove the entire arcuate nucleus (ARC) from the third slice (+2 to +5.5 mm).Brain tissue samples were frozen in liquid nitrogen and stored at −70 °C until analyzed.

2.7. NPY mRNA determination

Total RNA was extracted from the arcuate nucleus. The extraction was carried out using the guanidine thiocyanate–phenol–chloroform method (Chomczynski and Sacchi, 1987). Tissue samples were collected in guanidine thiocyanate with added β-mercaptoethanol, homogenized in this buffer and water saturated molecular biology grade phenol. Sodium lauroyl sarcosinate, 2 M sodium acetate, and chloroform were then added. After centrifugation, the aqueous phase was precipitated with the isopropanol, resuspended in guanidine thiocyanate buffer and reprecipitated with isopropanol. The RNA pellet was washed with 75% ethanol and stored at 70 °C in 100% ethanol. The RNA pellet was reconstituted in RNA storage buffer and total amount of RNA was measured using a spectrophotometer.

Samples were analyzed by the slot-blot method using nylon membranes (Zeta-Probe, BioRad, Hercules, CA). Aliquots of total RNA were dissolved in 7.4% formaldehyde: 6×SSC (1×SSC = 0.15 M NaCl, 0.015 M Na Citrate) and denatured for 10 min at 68 °C. Two μg of total RNA from each sample was slotted onto 6× SSC soaked nylon membrane (Zeta-Probe, BioRad, Hercules, CA) using a slot-blot apparatus (BioRad, Hercules, CA) attached to vacuum suction. The membranes were then placed under UV light and even loading of samples was verified by shadowing of the nucleic acids (Thurston and Saffer, 1989). The RNA was then fixed onto the nylon after air-drying by UV crosslinking using a stratalinker (model 1800, Stratagene, La Jolla, CA). The membrane was then prehybridized for 24 h at 42 °C in 50% formamide, 5× SSC, 10× Denhardt's solution, 0.1% SDS, and denatured salmon sperm DNA in 50 mM Na phosphate, pH 6.5. The hybridization procedure was carried out using a radiolabelled cDNA pBLNPY-1 probe that was grown in our laboratory and generously provided

by Dr. Steven L. Sabol, Laboratory of Biochemical Genetics, NHLBI, NIH Bethesda, Maryland. This cDNA contains a 511 base-pair sequence comprising most of the rat-prepro-neuropeptide Y gene. For the β-actin hybridization, we used β-actin probe obtained from ONCOR Inc. (Gaithersburg, MD). The hybridization medium (16 ml per tube) was 50% formamide, 5× SSC, 2× Denhardt's solution, 0.2% SDS, denatured salmon sperm DNA and yeast t-RNA in 50 mM Na phosphate, pH 6.5 with the addition of 10^6 cpm/ml of [^{32}-P]-dCTP (specific activity = 3000 Ci/mmol) random primer labeled probe. After hybridization for 72 h at 42 °C, the nylon membranes were subjected to a high and low salt washing and then placed in a cassette with Kodak XAR film for autoradiography in a −70 °C freezer.

2.8. NPY radioimmunoassay

PVN were homogenized in 1 ml of 1 M CH_3COOH/95% ethanol (20:80 vol/vol) in polypropylene tubes. The homogenates were centrifuged at 13,000 *g* for 15 min. The pellets were re-extracted with the same procedure and the two supernatants were combined, lyophilized and later used for rat NPY radioimmunoassay (NPY RIA) (Peninsula Laboratories, Inc., Belmont, CA). The NPY RIA kit was validated before use with tissue extracts. Dose–response curves for PVN tissue extracts and increasing concentrations of the NPY standard added to rat PVN tissue extracts were parallel ($p>0.05$) to the standard curve. NPY (ranging from 4 pg to 32 pg) added to rat PVN tissue extract was consistently recovered from 100 µl of extract (90–100%). The assay sensitivity was 16 pg/tube. The cross-reactivity test, provided by the Peninsula Laboratories, indicated a cross-reaction of less than 3% with human pancreatic polypeptide.

2.9. Leptin radioimmunoassay

Blood samples were centrifuged for 20 min at 2000 *g* and sera stored at −4 °C until use. On the day of the RIA, samples were slowly thawed and 100 µl sera added to the RIA tube for use in the rat leptin RIA kit (Linco Research Inc., St. Louis, MO). Standard concentrations ranged between 0.5 ng/ml and 50 ng/ml, which is within the limit of linearity. All samples were within this range. The assay sensitivity is 0.5 ng/ml (100 µl sample size). The cross-reactivity test, provided by Linco Research Inc., indicated no cross-reaction with insulin, glucagon or somatotropin release-inhibiting factor.

2.10. Specific experimental protocols

2.10.1. Experiment 1: feeding and NPY response to a one-time dose of 4 mg/kg BT

In Experiment 1, rats were randomly divided into 4 treatment groups (n = 8–9/group): 1) Saline s.c. with food allowed ad libitum; 2) Saline s.c. with food deprivation; 3) BT: 4 mg/kg s.c. with food allowed ad libitum; 4) BT: 4 mg/kg s.c. with food deprivation. Injections were carried out between 0800 h and 0900 h. Food intake was measured at 2, 3 and 6 h in the rats that had access to food. The food deprived rats had no access to food, and food was removed from their cages immediately prior to BT administration. Rats were sacrificed the same day after 6 h at 1400 h and tissues were harvested for analysis.

2.10.2. Experiment 2: feeding and NPY response to 2-day pump-infusion or repeated injections of 4 mg/kg BT

In Experiment 2, rats were randomly divided into 2 treatment groups: drug treatment by either peripheral pump infusion (0.0254 mg/µl/h × 10 µl/h BT) or by repeated s.c. injections (4 mg/kg BT every 6 h for 48 h starting at 0800 h on Day 1). The amount of BT contained in the pump was equivalent to a dose of 4 mg/kg every 6 h. Each group was further subdivided into 3 treatment groups (n = 5–8/group): 1) Saline with food allowed ad libitum; 2) BT with food allowed ad libitum; and 3) BT with food intake restricted to the level of that observed in the saline-treated rats. These pair-fed rats were given the average amount of food that the saline group ate at the previous time point. All groups were run simultaneously. Food intake was measured every 6 h for 48 h. Rats were sacrificed 1–2 h after the final set of injections (1000 h on Day 3), and tissues were harvested for analysis.

2.10.3. Experiment 3: feeding and NPY response to repeated injections of ascending doses of BT

In Experiment 3, rats were divided into 3 treatment groups (n = 10/group): 1) Saline s.c. with food allowed ad libitum; 2) BT s.c. with food allowed ad libitum; and 3) BT s.c. with food intake restricted to that of the saline-treated rats. The pair-fed rats were given the average amount of food that the saline group ate at the previous time point. All groups were run simultaneously. The injections were given every 6 h for 24 h. To overcome potential tachyphylaxis, the amount of BT given was increased with each injection such that the doses given at 1, 6, 12, 18 and 24 h were 1, 2, 4, 8 and 16 mg/kg respectively. The final injection occurred at 0800 h. Food intake was measured at each injection time-point, at 1, 6, 12, 18 and 24 h, and additionally at 2 h after the first injection (thus at the 0, 2, 6, 12, 18 and 24 h time points). Rats were sacrificed at 1000 h and tissues taken for analysis.

2.10.4. Experiment 4: effect of BT (4 mg/kg dose) on serum leptin in food-deprived and non-deprived rats

In Experiment 4, rats were randomly divided into 4 treatment groups (n = 8/group): 1) Ad libitum access to chow overnight followed by saline s.c.; 2) Ad libitum access to chow overnight followed by BT (4 mg/kg) s.c.; 3) Food deprived overnight followed by saline s.c.; 4) Food deprived overnight followed by BT (4 mg/kg) s.c. Injections were administered at 0800 h, at which time, all the chow were taken from all rats, and 6 h later, rats were sacrificed and blood was collected for serum leptin determination.

2.11. Statistical analyses

Experiment 1 Food intake data were analyzed using t-tests and a Bonferroni correction was applied for all pair-wise comparisons to control for Type I error. Data are presented as mean ± SEM.

Experiment 2 Food intake data were analyzed using 2-way ANOVAs. For treatments showing a significant interaction, t-tests were conducted in order to examine specific differences. The food intake of the pair-fed groups was not included in the analysis as they were not presented with food for the first 6 h. A Bonferonni correction was applied for all pair-wise comparisons to control for Type I error. Data are presented as mean ± SEM.

Experiment 3 Food intake data were analyzed using repeated measures ANOVA, followed by t-tests. The intake of the pair-fed rats was not used for data analysis purposes as they were only presented with food 6 h after the start of the drug administration. A Bonferonni correction was applied for all pair-wise comparisons to control for Type I error. Data are presented as mean ± SEM

Experiment 4 Data were analyzed for main effects by a 2 factor ANOVA: drug (saline or BT) × food intake status (ad lib or deprived). For treatments showing a main effect by ANOVA, means were compared by multiple comparison contrasts. Data are presented as mean ± SEM.

Experiments 1–3 NPY mRNA and peptide data were analyzed for main effects by a one-factor ANOVA. For treatments showing a main effect by ANOVA, means were compared by post-hoc analysis of the data using Fisher's protected least significant difference *t*-test. Data are presented as mean ± SEM.

3. Results

3.1. Experiment 1: feeding and NPY response to a one-time dose of 4 mg/kg BT

In this experiment, feeding and NPY response (mRNA and peptide) to a one time dose of 4 mg/kg BT, s.c. were measured. There was a significant effect of BT ($t(15)=6.65$, $p<0.001$; Fig. 1) on food intake at the 2–3 h interval. Food intake measured during the 0–2 h interval and the 3–6 h interval was non-significantly greater in the BT treated rats (0–2 h interval: $t(15)=0.907$, $p>0.05$, $t(15)=3.83$, $p>0.05$). NPY mRNA and NPY peptide were not significantly different among groups ($F_{1,30}=1.904$, $p>0.05$; $F_{1,28}=0.828$, $p>0.05$; Table 1). In a regression analysis including all groups, there were no significant correlations between NPY peptide or NPY mRNA and food intake. However, the correlation between the cumulative intake at the 6 h time point and NPY mRNA revealed a trend towards significance ($F_{1,14}=3.752$, $p=0.073$, $R=0.460$).

3.2. Experiment 2: feeding and NPY response to 2 day pump-infusion or repeated injections of 4 mg/kg BT

In this experiment, feeding during either a 2-day pump-infusion or repeated injections of 4 mg/kg BT was measured. At the 0–6 h time point, there was an effect of drug treatment, such that BT treated rats consumed significantly more food as compared with the saline treated rats ($F_{3,28}=81.41$, $p<0.0001$; Fig. 2). Analysis of the food intake over the first 24 h showed a significant effect of route of administration ($F_{3,28}=7.01$, $p<0.05$), but not drug treatment ($F_{3,28}=2.196$, $p>0.05$). Follow-up t-tests revealed that the saline-pump group had a significantly lower 0–24 h food intake, than the saline-injection group ($t(14)=3.00$, $p<0.001$). Additionally BT-pump treated rats had higher 24 h food intake than saline-pump treated rats ($t(14)=2.772$, $p<0.05$). This difference did not persist over the next 24 h measurement (i.e. Day 2 cumulative intakes were not significantly different between the saline vs. BT treated groups, regardless of route of administration of the drug treatment; $F_{3,28}=0.072$, $p>0.05$). The "pair-fed or yoked" groups' intakes are not represented in Fig. 2 as they were given access to chow 2 h after the other groups; so their data as a means of comparison for the behavioral tests isn't particularly meaningful. However the molecular aims of the experiment required their use as control groups. In a regression analysis containing all groups, NPY mRNA levels were not significantly correlated with food intake at any time point (Table 1).

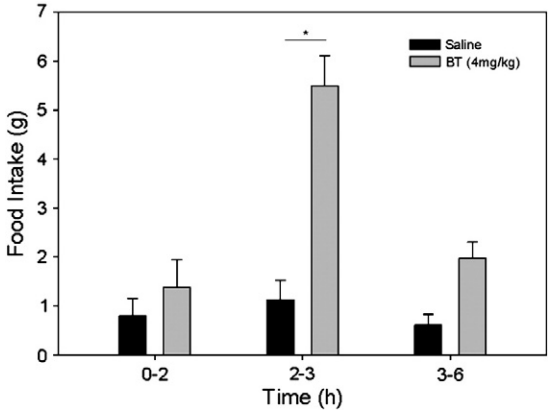

Fig. 1. Food intake following a single dose of butorphanol (BT, 4 mg/kg). *P<0.001. N = 8–9/group. Values are presented as means ± SEM.

3.3. Experiment 3: feeding and NPY response to repeated injections of ascending doses of BT

In this experiment, feeding and NPY response to repeated injections of ascending doses of BT was measured. The repeated measures ANOVA yielded a significant interaction ($F_{4,15}=7.265$, $p<0.01$). T-tests revealed a significant effect of BT treatment at the 1–6 h time point; ($t(18)=4.40$, $p<0.0001$). No significant differences in food intake were found at the other time points (Fig. 3). Cumulative intake at 24 h was significantly greater in the BT treated rats, as compared with the saline treated rats ($p<0.001$). The "pair-fed or yoked" groups' intakes are not represented in Fig. 3 as they were given access to chow 1 h after the other groups; so their data as a means of comparison for the behavioral tests is not meaningful. However the molecular aims of the experiment required their use as control groups. NPY mRNA levels were significantly higher in the saline treated rats ($F_{2,27}=3.506$, $p<0.05$) (Table 1). In a regression analysis including all groups, NPY mRNA levels and food intake at 0–12 and 0–18 h were inversely correlated: $F_{1,28}=6.274$, $p=0.018$, $R=0.428$; and $F_{1,28}=3.829$, $p=0.0604$, $R=0.346$ respectively.

3.4. Experiment 4: effect of BT (4 mg/kg dose) on serum leptin in food-deprived and non-deprived rats

In this experiment, serum leptin was measured following BT administration in food deprived and non-deprived rats. There was a main effect of food status (deprived or not-deprived) on serum leptin ($F_{1,28}=41.873$, $p<0.0001$; Fig. 4). There was no main effect of BT on serum leptin ($F_{1,28}=0.325$, $p>0.05$), and no interaction between food status or BT treatment ($F_{1,28}=0.479$, $p>0.05$).

4. Discussion

The present data suggest that BT has robust effects on feeding that are dependent on the dosing schedule. An acute single dose (4 mg/kg) results in a powerful feeding response. However, with repeated injections of the same dose of BT within the same day, the magnitude of the feeding response wanes and falls to a constant level. Additionally, with repeated injections of the same dose of BT, the cumulative long-term intake of BT-treated rats did not differ from that of control rats, indicating that the rats compensate for the increased feeding following BT injections by decreased feeding at a later time. The only paradigm in which BT injections resulted in additional feeding stimulation was with a repeated ascending dose schedule, although the cumulative increase was not significantly different from the saline condition. This might suggest that BT-treated rats may undergo tachyphylaxis and become less sensitive to BT stimulation, alternatively it is possible that the effect of BT was obscured by the normal increase in feeding by the saline rats during the dark period. Another possibility is that the higher doses of BT did not significantly increase interval food intake (i.e. intake at 18–24 h and 24–30 h; the time periods following the 8 mg/kg and 16 mg/kg BT doses) due to the possible sedative effects of BT at these higher doses. The sedative effects of opiates and opioids (Rudski et al., 1992) as well as the development of tolerance to these effects after repeated injections (Morley et al., 1982; Thornhill et al., 1979) have been noted in other studies.

Opioids have been extensively studied in relation to food intake. Their primary mode of action in stimulating food intake is thought to be by enhancing the rewarding properties of food (Bodnar, 2004). For example, injection of opioid agonists into the nucleus accumbens (Pritchett et al., 2010), central nucleus of the amygdala (CeA; Levine et al., 2004b), ventral tegmental area (Badiani et al., 1995), prefrontal cortex (Mena et al., 2011) and PBN (Wilson et al., 2003) (areas associated with taste, reward and emotional processing) has been found to stimulate feeding. Peripheral BT administration (4 mg/kg) has been shown to stimulate c-fos immunoreactivity in

Table 1
NPY mRNA (O.D. units; optical density) and peptide (pg/ml) measures obtained in the 3 experiments. Experiment 1, NPY response (mRNA and peptide) to a one time dose of 4 mg/kg BT. N = 8–9 per group. Experiment 2, NPY mRNA response to 2 day pump-infusion or repeated injections of 4 mg/kg BT. N = 5–8 per group. Experiment 3, NPY response to repeated injections of ascending doses of BT. N = 10 per group.

Experiment 1			Experiment 2		Experiment 3	
Treatment	NPY mRNA (O.D. units)	NPY peptide (pg/ml)	Treatment	NPY mRNA (O.D. units)	Treatment	NPY mRNA (O.D. units)
Saline *ad lib food*	2.3 ± 0.3	66.2 ± 5.7	Saline[1]	10.0 ± 0.6	Saline	6.3 ± 0.4
BT (4 mg/kg) *ad lib food*	2.1 ± 0.5	54.9 ± 5.6	BT[1]	9.3 ± 0.6	BT	4.7 ± 0.5*
Saline no food	2.8 ± 0.6	62.5 ± 4.1	BT restrict[1]	8.8 ± 1.4	BT — pair fed	5.9 ± 0.4
BT (4 mg/kg) no food	1.9 ± 0.1	66.2 ± 5.7	Pump saline	9.0 ± 0.7	Route of administration: subcutaneous.	
Route of administration: subcutaneous.			Pump BT	9.1 ± 1.1	*p<0.05. compared between all groups'	
BT dose: 4 mg/kg.			Pump BT restrict	9.7 ± 0.6	BT dose: 1, 2, 4, 8, and 16 mg/kg administered at 0, 6, 12, 18 and 24 h.	
			Route of administration: [1]subcutaneous.			
			BT dose: 4 mg/kg every 6 h for 48 h.			

the PVN, CeA, and nucleus of the solitary tract (NTS), however only the PVN administration (30 μg) of BT stimulated food intake (Kim et al., 2001). The absence of an effect of BT when injected into the CeA may be a consequence of its more complicated receptor profile.

It is known that NPY is among the most potent central orexigenic peptides and there is a large body of research that shows that the ability of NPY to potently stimulate food intake is partly dependant on mu- and kappa- opioid receptors (Israel et al., 2005). Based on the known opioid/NPY interactions in the control of feeding, we hypothesized that BT effects may be mediated through NPYergic pathways, yet NPY gene expression in the ARC was not influenced by BT independently. In all experiments in which NPY gene expression was measured, the amount of food consumed and the level of NPY mRNA were inversely correlated: rats consuming greater than control levels of food had decreased NPY levels and those consuming less had increased NPY mRNA levels. Thus while NPY gene expression was affected by feeding status, it was not affected by BT. It is possible that NPY release in PVN was affected by BT, but that was not measured in this study. Melanotan (MTII) administration (which is a melanocortin receptor (MCR) agonist) has been reported to produce a decrease in food intake and subsequent weight loss in mice, however prolonged administration results in tachyphylaxis, which is thought to be due to upregulation of NPY and AGRP mRNA (Bluher et al., 2004). This was in contrast to our findings, where we did see a tachyphylactic effect of BT administration, without the subsequent changes in NPY mRNA expression. It is possible that the regulation of NPY is more sensitive to anorexigenic agents, rather than orexigenic agents. That we could observe changes in NPY mRNA in the time-frame of the study indicates that there was enough time for BT to induce gene expression changes. Therefore, these data suggest, but do not definitively prove, that the strong BT feeding effect does not appear to be mediated by alterations in endogenous NPY.

Due to the robust feeding effects elicited by BT, we considered whether leptin levels were altered following BT administration. Again, like NPY, while feeding status altered leptin serum levels, BT treatment did not (Fig. 4). It has been very clearly demonstrated that leptin levels are altered when NPY levels are altered (Sainsbury et al., 1996, Schwartz et al., 1996). Thus it stands to reason that as BT did not alter NPY levels, leptin levels would perhaps also remain unaltered. In the present set of experiments, we saw that in the absence of altered food intake, leptin levels also did not vary between saline and butorphanol treated animals. This result is similar to what we found with NPY, where feeding status, and not BT treatment, resulted in alterations in NPY gene expression. Although previous work (Kotz et al., 1993; Kotz et al., 1995) has shown NPY/opioid interactions in feeding regulation, the present data suggest that BT does not exert its robust feeding effect through either of these mechanisms. In summary, the present data suggest that BT is a powerful feeding stimulant, which does not appear to operate through NPY or leptin pathways. The feeding produced by BT is sensitive to dose and the dosing paradigm. While peripheral administration of BT induces a more potent feeding response as compared with central administration, its effect is more robust than most other opioid receptor agonists (Levine and Morley, 1983; Morley et al., 1985). The melanocortins are a future candidate for investigating

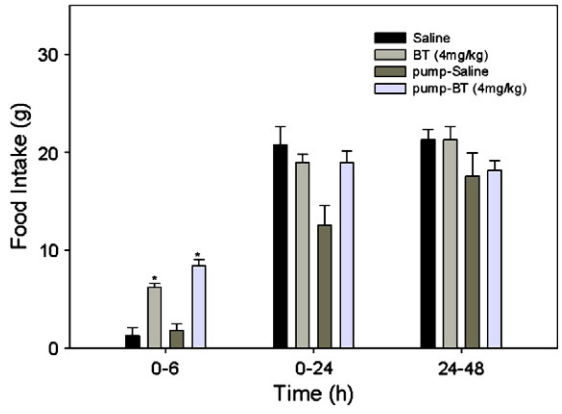

Fig. 2. Food intake following pump-infusion or repeated injections of butorphanol (BT, 4 mg/kg) over 48 h. At the 0–6 h time point BT treated rats consumed significantly more food as compared with the saline treated rats. *P<0.001. At the 0–24 h time point, the saline-injection group consumed significantly more food than the saline-pump group. P<0.001, and the BT-pump group also consumed significantly more food than the saline-pump group. P<0.05. N = 5–8/group. Values are presented as means ± SEM.

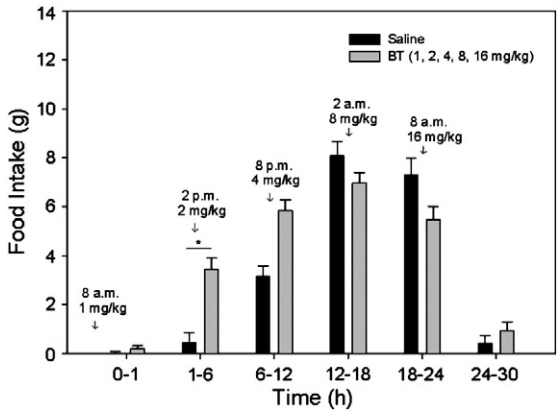

Fig. 3. Food intake following repeated injections of ascending doses of butorphanol (BT, 1, 2, 4, 8 and 16 mg/kg administered at 0, 6, 12, 18 and 24 h time points). At the 1–6 h time point BT treated rats consumed significantly more food as compared with saline treated rats. *P<0.0001. N = 10/group. Values are presented as means ± SEM.

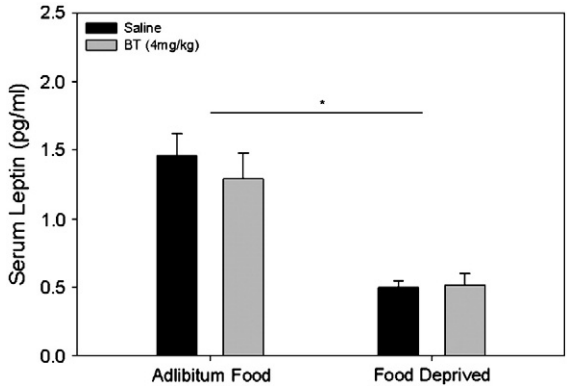

Fig. 4. Serum leptin levels following butorphanol (BT, 4 mg/kg) administration in food deprived and non-deprived rats. *P<0.001. N = 8/group. Values are presented as means ± SEM.

the mechanism via which BT exerts its potent orexigenic effects. Alpha-Melanocyte stimulating hormone (α-MSH) has potent anorexigenic effects, and it is also known to be involved in reducing morphine analgesia; for review see Reece (2010). It is possible that BT treatment may be inhibiting the anorexigenic activity of α-MSH, thus resulting in the over-feeding that we and others (Levine et al., 1994; Levine and Morley, 1983) have observed. Alternatively, it is possible that BT administration stimulates ghrelin secretion, which may explain the increased feeding observed. Elucidation of the pathways that are influenced by BT may provide key information pertinent to the role of the opioid receptors in feeding, and further investigation into this area may shed new light on the physiologic mechanism behind appetite control.

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References

Badiani A, Leone P, Noel MB, Stewart J. Ventral tegmental area opioid mechanisms and modulation of ingestive behavior. Brain Res 1995;670:264–76.
Bluher S, Ziotopoulou M, Bullen Jr JW, Moschos SJ, Ungsunan L, Kokkotou E, et al. Responsiveness to peripherally administered melanocortins in lean and obese mice. Diabetes 2004;53:82–90.
Bodnar RJ. Endogenous opioids and feeding behavior: a 30-year historical perspective. Peptides 2004;25:697–725.
Boggiano MM, Chandler PC, Viana JB, Oswald KD, Maldonado CR, Wauford PK. Combined dieting and stress evoke exaggerated responses to opioids in binge-eating rats. Behav Neurosci 2005;119:1207–14.
Chang KJ, Hazum E, Cuatrecasas P. Novel opiate binding sites selective for benzomorphan drugs. Proc Natl Acad Sci USA 1981;78:4141–5.
Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 1987;162:156–9.
Figlewicz DP. Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. Am J Physiol Regul Integr Comp Physiol 2003;284:R882–92.
Figlewicz DP, Bennett JL, Naleid AM, Davis C, Grimm JW. Intraventricular insulin and leptin decrease sucrose self-administration in rats. Physiol Behav 2006;89:611–6.
Gosnell BA, Levine AS. Reward systems and food intake: role of opioids. Int J Obes (Lond) 2009;33(Suppl 2):S54–8.
Israel Y, Kandov Y, Khaimova E, Kest A, Lewis SR, Pasternak GW, et al. NPY-induced feeding: pharmacological characterization using selective opioid antagonists and antisense probes in rats. Peptides 2005;26:1167–75.
Kim EM, Shi Q, Olszewski PK, Grace MK, O'Hare E, Billington CJ, et al. Identification of central sites involved in butorphanol-induced feeding in rats. Brain Res 2001;907:125–9.
Kim EM, Welch CC, Grace MK, Billington CJ, Levine AS. Chronic food restriction and acute food deprivation decrease mRNA levels of opioid peptides in arcuate nucleus. Am J Physiol 1996;270:R1019–24.
Kotz CM, Grace MK, Billington CJ, Levine AS. The effect of norbinaltorphimine, beta-funaltrexamine and naltrindole on NPY-induced feeding. Brain Res 1993;631:325–8.
Kotz CM, Grace MK, Briggs J, Levine AS, Billington CJ. Effects of opioid antagonists naloxone and naltrexone on neuropeptide Y-induced feeding and brown fat thermogenesis in the rat. Neural site of action. J Clin Invest 1995;96:163–70.
Kotz CM, Grace MK, Briggs JE, Billington CJ, Levine AS. Naltrexone induces arcuate nucleus neuropeptide Y gene expression in the rat. Am J Physiol 1996;271:R289–94.
Lahti RA, Mickelson MM, McCall JM, Von Voigtlander PF. [3H]U-69593 a highly selective ligand for the opioid kappa receptor. Eur J Pharmacol 1985;109:281–4.
Lambert PD, Wilding JP, al-Dokhayel AA, Gilbey SG, Ghatei MA, Bloom SR. Naloxone-induced anorexia increases neuropeptide Y concentrations in the dorsomedial hypothalamus: evidence for neuropeptide Y–opioid interactions in the control of food intake. Peptides 1994;15:657–60.
Levine AS, Grace M, Portoghese PS, Billington CJ. The effect of selective opioid antagonists on butorphanol-induced feeding. Brain Res 1994;637:242–8.
Levine AS, Jewett DC, Cleary JP, Kotz CM, Billington CJ. Our journey with neuropeptide Y: effects on ingestive behaviors and energy expenditure. Peptides 2004a;25:505–10.
Levine AS, Morley JE. Butorphanol tartrate induces feeding in rats. Life Sci 1983;32:781–5.
Levine AS, Olszewski PK, Mullett MA, Pomonis JD, Grace MK, Kotz CM, et al. Intra-amygdalar injection of DAMGO: effects on c-Fos levels in brain sites associated with feeding behavior. Brain Res 2004b;1015:9-14.
Mena JD, Sadeghian K, Baldo BA. Induction of hyperphagia and carbohydrate intake by mu-opioid receptor stimulation in circumscribed regions of frontal cortex. J Neurosci 2011;31:3249–60.
Morley JE, Levine AS, Grace M, Kniep J. An investigation of the role of kappa opiate receptor agonists in the initiation of feeding. Life Sci 1982;31:2617–26.
Morley JE, Parker S, Levine AS. Effect of butorphanol tartrate on food and water consumption in humans. Am J Clin Nutr 1985;42:1175–8.
Niwa M, Nose T, Nozaki M, Tsurumi K, Fujimura H. Effects of butorphanol and its metabolites on the levels of monoamines and their metabolites in the rat brain. Jpn J Pharmacol 1985;39:515–28.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 2nd ed. Sydney; Orlando: Academic Press; 1986.
Pritchett CE, Pardee AL, McGuirk SR, Will MJ. The role of nucleus accumbens adenosine–opioid interaction in mediating palatable food intake. Brain Res 2010;1306:85–92.
Reece AS. Hypothalamic opioid -melanocortin appetitive balance and addictive craving. Med Hypotheses 2010;76:132–7.
Rudski JM, Schaal DW, Thompson T, Cleary J, Billington CJ, Levine AS. Effects of methadone on free feeding in satiated rats. Pharmacol Biochem Behav 1992;43:1033–7.
Sainsbury A, Cusin I, Doyle P, Rohner-Jeanrenaud F, Jeanrenaud B. Intracerebroventricular administration of neuropeptide Y to normal rats increases obese gene expression in white adipose tissue. Diabetologia 1996;39:353–6.
Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, et al. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996;45:531–5.
Thornhill JA, Hirst M, Gowdey CW. Changes in core temperature and feeding in rats by levorphanol and dextrorphan. Can J Physiol Pharmacol 1979;57:1028–32.
Thurston SJ, Saffer JD. Ultraviolet shadowing nucleic acids on nylon membranes. Anal Biochem 1989;178:41–2.
Walsh SL, Chausmer AE, Strain EC, Bigelow GE. Evaluation of the mu and kappa opioid actions of butorphanol in humans through differential naltrexone blockade. Psychopharmacology (Berl) 2008;196:143–55.
Wilson JD, Nicklous DM, Aloyo VJ, Simansky KJ. An orexigenic role for mu-opioid receptors in the lateral parabrachial nucleus. Am J Physiol Regul Integr Comp Physiol 2003;285:R1055–65.