Order Number 9224790

Free feeding and intake following regulatory challenges in rats: Roles of selective opioid receptor subtype antagonists

Arpune, Dulmanic, Ph D

City University of New York, 1992

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Ann Arbor, MI

Free Feeding and Intake Following Regulatory Challenges in Rats: Roles of Selective Opioid Receptor Subtype Antagonists

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1992

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satifaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

Free Feeding and Intake Following Regulatory Challenges in Rats: Roles of Selective Opioid Receptor Subtype Antagonists

by

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The present studies evaluated the central effects of the specific antagonists, beta-funaltrexamine (ß-FNA), nor-binaltorphimine (Nor-BNI) and [D-Ala^2, Leu^5, Cys^6]-Enkephalin (DALCE), upon ingestive behavior, specifically under such conditions as spontaneous intake, food deprivation, 2DG glucoprivation, palatable intake and nocturnal feeding. ß-FNA is a non-equilibrium, long-term antagonist of the mu receptor and a reversible, short-term agonist of the kappa opioid receptor. Nor-BNI is a highly-selective kappa receptor antagonist with a 170-fold greater affinity for kappa relative to mu receptors. DALCE acts as a short-acting delta, and secondarily mu, agonist and as a long-acting antagonist at the delta receptor. In short-term intake tests, food intake was significantly increased by ß-FNA (1-20µg, i.c.v.) for up to 6h and by DALCE (10µg, i.c.v.) for up to 10h. Short-term ß-FNA hyperphagia was reduced by the kappa antagonist, Nor-BNI, but not by mu antagonism with ß-FNA, suggesting kappa receptor involvement. Similarly, short-term DALCE hyperphagia was eliminated by general opiate (naltrexone: NTX) and kappa (Nor-BNI) antagonism, but was minimally affected by mu (ß-FNA) and delta (DALCE) antagonism, indicating that the kappa receptor is in the final common path of this response. Whereas long-term ß-FNA (10-20µg) treatment significantly inhibited free feeding 24, 48 and 72h following injection, DALCE

(40μg) only minimally decreased food intake and body weight gain after 24-96h. The increased intake demonstrated following 24h of food deprivation was significantly suppressed (33-49%) following β-FNA (10-20μg) pretreatment. In contrast, DALCE, administered prior to food deprivation (24h), failed to affect subsequent 24h intake and only sporadically decreased food intake and body weight after 48 and 72h. Nocturnal intake, assessed two hours into the dark cycle, was significantly inhibited by central pretreatment (1h) with Nor-BNI (20μg, 53-54%) and NTX (20μg, 47-60%). Such evidence indicate that the antagonists display differential response in free feeding (NTX=Nor-BNI>β-FNA>>DALCE) and in deprivation-induced feeding (NTX=β-FNA>Nor-BNI>>DALCE). The hyperphagia induced by the anti-metabolic glucose analogue, 2-deoxy-D-glucose, was significantly inhibited by central β-FNA (10-20μg, 75-100%) pretreatment (24h), Nor-BNI (1-20μg, 33-79%) and NTX (20μg, 40-68%). DALCE (10μg) pretreatment(24h), on the other hand, only transiently (2h) decreased glucoprivic intake. Similarly, the short-term hyperphagia observed following exposure to a high-fat diet was significantly attenuated by central Nor-BNI (1-20μg, 33-79%) and NTX (20μg, 47-51%) while significantly potentiated by long-term DALCE (1μg) pretreatment. These data also indicate that the antagonists exhibit differential response to glucoprivic (NTX=β-FNA>Nor-BNI>>DALCE) and palatable (NTX=Nor-BNI>β-FNA>>DALCE) intakes. Essentially, the opioid receptor subtypes are involved in regulating various forms of ingestive behavior, addressing issues pertaining to obesity, regulatory challenges and palatability.

Acknowledgements

I wish to express my deepest appreciation and gratitude to Dr. Richard J. Bodnar, who has been most instrumental in my work as a graduate student. The guidance he has provided would benefit me throughout my professional life.

I would also like to thank my parents for their support and tolerance.

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Introduction

Recent development of highly-selective antagonists for the different opioid receptor subtypes has provided a means to characterize precisely the physiological functions of each opioid subtype in ingestive behavior. Biochemical and pharmacological evidence have indicated the existence of distinct opioid receptor subtypes termed mu1, mu2, kappa, delta, sigma and epsilon (Lord, Waterfield, Hughes & Kosterlitz, 1977; Martin, Eades, Thompson, Huppler & Gilbert, 1976; Pasternak & Wood, 1986; Schulz, Faase, Wuster & Herz, 1979). These receptors were shown to play a significant role in a number of behaviors, including analgesia, tail pinch-induced feeding and learning and memory processes (Morley, Levine, Yim & Lowy, 1983a; Riley, Zellner & Duncan, 1980). The mu receptors have been specifically implicated in supraspinal analgesia, respiratory depression, inhibition of gastrointestinal transit, bradycardia, prolactin release, dopamine turnover and some components of physical dependence (Pasternak & Wood, 1986). The kappa receptor has been implicated in spinal analgesia and sedation (Akil, Watson, Young, Lewis, Khachaturian & Walker, 1984). The delta receptor appears to mediate both spinal and supraspinal analgesia, growth hormone release, dopamine turnover and reversal of endotoxic shock syndrome (Akil et al., 1984). Further, the sigma and the epsilon receptors are responsible for a multiplicity of behaviors, such as analgesia, locomotor stimulation and tachycardia. However, since the sigma receptor is not a true receptor (Zukin & Zukin, 1981) and since the epsilon receptor is as yet poorly characterized (Schulz et al., 1979), the effects of the mu, kappa and delta receptors will be evaluated in ingestive behavior. Typically, opioid receptor agonists stimulate food intake under various ingestive conditions, while general opioid receptor antagonists inhibit

food intake (Morley et al., 1983a). The general non-specific opiate receptor antagonists, naloxone and naltrexone, attenuate hyperphagia and hyperdipsia following deprivation, hyperphagia induced by either 2-deoxy-D-glucose (2DG) or insulin glucoprivation, nocturnal intake and intake following introduction of palatable diets (Morley et al., 1983a). Similarly, beta-chlornaltrexamine (ß-CNA), a long-term, non-specific antagonist, reduces spontaneous feeding, body weight gain and feeding induced by selective agonists of mu, kappa and delta receptors (Gosnell, Grace & Levine, 1987). The inability of these general antagonists to distinguish among specific receptor subtypes led to the development of highly-selective antagonists. Beta-funaltrexamine (ß-FNA), the fumarate methyl ester of naltrexone, binds reversibly as a short-term agonist at the kappa receptor, and binds irreversibly as a long-term antagonist at the mu receptor (Portoghese, Larson, Sayre, Fries & Takemori, 1980; Takemori, Larson & Portoghese, 1981). ß-FNA inhibited spontaneous intake following ventricular administration (Ukai & Holtzman, 1988) and deprivation intake following hypothalamic paraventricular nucleus (PVN) injections (Levine, Grace, Billington & Portoghese, 1989). Nor-binaltorphimine (Nor-BNI) is a potent, though reversible, kappa receptor antagonist, binding with a 170-fold greater affinity for kappa relative to mu receptors (Portoghese, Lipkowski & Takemori, 1987; Takemori, Ho, Naeseth & Portoghese, 1988). Central pretreatment with Nor-BNI blocks intake induced by food deprivation and by opioid receptor agonists (Levine, Grace, Billington & Portoghese, 1990). [D-Ala^2,Leu^5,Cys^6]-enkepholin (DALCE) displays high affinity for the delta receptors, moderate affinity for mu receptors and negligible affinity for kappa receptors. While DALCE readily dissociates from the mu receptors, it fails to do so from the delta receptors where it convalently binds. Thus, DALCE is a short-acting delta and secondarily mu agonist and a long term, selective delta antagonist

(Bowen, Hellewell, Kelemen, Huey & Stewart, 1987).

The present series of experiments evaluated these specific opioid receptor subtype antagonists for their abilities to alter food intake under various feeding conditions, including free feeding, regulatory challenges such as deprivation and 2DG glucoprivation, palatability and nocturnal situations to determine which opioid receptor subtypes mediated which different forms of ingestive behavior. The following sections provide background information pertaining to: A) opioid peptides and endogenous opioid families, B) specific opiate receptor subtypes, C) ingestive behavior, D) opioid peptides and ingestive behavior, and E) a rationale for the present experiments.

A. Opioid Peptides and Endogenous Opioid Families

Biochemical and pharmacological actions of opiates were characterized by the high degree of structural and steric specificity bound to vertebrate membranes (Simon, Hiller & Edelman, 1973). Saturability and stereospecificity were demonstrated in the rodent brain with the levo-rotatory, rather than the dextro-rotatory, isomer being the active component of both natural and synthetic opiates (Terenius, 1973). Opiate receptor binding is restricted exclusively to both central and peripheral nervous tissue, exerting its effects primarily through synaptic transmission (Gray & Whittaker, 1962; Pert, Snowman & Snyder, 1974; Pert & Snyder, 1973 a,b; Terenius, 1973). Regional variations of opiate binding in the brains of rat, monkey and human were demonstrated with the highest density of binding occurring in the anterior portion of the cingulate gyrus, anterior hypothalamus, thalamus, periaqueductal gray, substantia gelatinosa of the spinal cord and the limbic system (Hiller, Pearson & Simon, 1973; Kuhar, Pert & Snyder, 1973; Simon, 1975). Opiate binding was also identified in certain smooth muscles of the peripheral nervous system. Opiate agonists inhibit electrically-induced contractions of both the myenteric plexus and the longitudinal muscle of the guinea-pig ileum (GPI) as well as the mouse vas deferens (MVD). Opiates decrease acetylcholine release following nerve stimulation, and opiate antagonists reverse this effect (Cox & Weinstock, 1966; Creese & Snyder, 1975; Hughes, 1975a; Hughes, Kosterlitz & Leslie, 1975a; Paton, 1957; Pert & Snyder, 1973a).

The identification of opiate receptors in the brain, guinea-pig ileum and mouse vas deferens suggested the presence of endogenous ligands for these receptors. Several studies found evidence for the existence of opiate-like substances. Acid extracts from whole rat brain and cerebrospinal fluid

inhibited binding of dihydromorphine to membrane-bound opiate receptors (Terenius and Wahlstrom, 1974; Terenius and Wahlstrom, 1975). This interaction was reversible as well as competitive in nature. Hughes and Kosterlitz isolated a substance with pharmacological properties similar to morphine which inhibited contraction of the mouse vas deferens following stimulation of the intramural nerves (Henderson, Hughes & Kosterlitz, 1972; Hughes, 1975a,b; Hughes et al., 1975a). Furthermore, crude preparations from the bovine and porcine pituitary glands and the rat brain led to the isolation of a typical opiate agonist (Cox, Opheim, Teschemacher & Goldstein, 1975; Pasternak, Goodman & Snyder, 1975; Teschemacher, Opheim, Cox & Goldstein, 1975).

In 1975, Hughes and his colleagues reported the structures of two opioid pentapeptides, methionine-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH) and leucine-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH), from the pig and cow brain (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975b; Hughes, Smith, Morgan & Fothergill, 1975c). Both peptides had potent agonist activity at opiate receptor sites, producing a dose-related inhibition of electrically evoked contractions of the guinea-pig ileum and the vas deferens which were antagonized by naloxone (Hughes et al., 1975a; Kosterlitz & Watt, 1968). Met-enkephalin is twenty times more active than normorphine in the mouse vas deferens and equipotent with normorphine in the guinea-pig ileum. In contrast, leu-enkephalin possessed half the potency of that of the met-enkephalin in the mouse vas deferens, but only one-fifth of the potency of met-enkephalin in the guinea-pig ileum. The amino acid sequence of met-enkephalin was located at positions 61-65 of Beta-lipotropin (ß-LPH), a large peptide containing 91 amino acids that was isolated from the anterior pituitary glands of the sheep (Li, 1964; Li, Barnafi, Chretien & Chung, 1965), pig (Graf, Barat, Cseh & Sajgo,

1971) and man (Cseh, Barat, Patthy & Graf, 1972). Also identified was beta-endorphin (61-91), the terminal carboxy fragment of ß-LPH (Li & Chung, 1976) which, unlike ß-LPH, possessed potent opioid activity *in vitro* and *in vivo* (Cox, Goldstein & Li, 1976; Bradbury, Smyth, Snell, Birdsall & Hulme, 1976). Two other opiate-like peptides, alpha-endorphin (ß-LPH: 61-76) and gamma-endorphin (ß-LPH: 61-77) were isolated from the porcine hypothalamus and posterior pituitary (Guillemin, Ling & Burgus, 1976; Ling, Burgus & Guillemin, 1976).

Within the central nervous system, ß-endorphin is found in high concentrations in the arcuate nucleus of the medial basal hypothalamus and anterior and intermediate pituitary where it is synthesized as part of a 31,000 molecular weight protein ("pro-opiocortin") (Bloch, Bugnon, Fellman & Lenys, 1978; Bloom, Battenberg, Rossier, Ling, Leppaluoto, Vargo & Guillemin, 1977; Nilaver, Zimmerman, Defendini, Liotta, Krieger & Brownstein, 1979; Pelletier, Leclerc, LaBrie, Cote, Chretien & Lis, 1977; Watson, Barchas & Li, 1977). Pro-opiomelanocortin (POMC), the precursor molecule from which ß-LPH/ß-endorphin molecules is derived, also gives rise to adrenocorticotropin hormone (ACTH: 1-39) which is further cleaved into alpha-melanocyte-stimulating hormone (alpha-MSH: [N-acetyl ACTH-(1-13)-NH_2]) and corticotropin-like intermediate lobe peptide (CLIP: ACTH (18-39)) (Brownstein, 1980; Eipper & Mains, 1978; Li & Chung, 1976; Mains, Eipper & Ling, 1977; Roberts & Herbert, 1977). The amino terminus of the POMC gene contained an active ACTH/MSH core, namely gamma-melanocyte-stimulating hormone (gamma-MSH) (Eipper & Mains, 1980; Ling, Ying, Minick & Guillemin, 1979).

Besides the ß-lipotropin molecule, a number of larger molecular weight peptides containing the met-enkephalin and leu-enkephalin sequence have been isolated. Lewis and his colleagues identified a 50,000 molecular weight

precursor protein molecule (proenkephalin) from the adrenal gland which contains met-enkephalin and leu-enkepholin sequences in a ratio of about 7 to 1 (Kimura, Lewis, Stern, Rossier, Stein & Udenfriend, 1980; Lewis, Stein, Gerber, Rubenstein & Udenfriend, 1978). Neural pathways containing proenkephalin are distributed widely throughout the central and peripheral nervous systems, and in contrast to the ß-endorphin/ACTH molecule, all of the active peptides of the proenkephalin molecule are opioid in nature (Elde, Hokfelt, Johansson & Terenius, 1976; Hokfelt, Elde, Johansson, Terenius & Stein, 1977; Hughes, Kosterlitz & Smith, 1977; Mizuno, Minamino, Kangawa & Matsuo, 1980).

Prodynorphin is the third precursor molecule that was identified in areas such as the brain stem, hypothalamus, posterior pituitary and gut (Goldstein, Fischli, Lowney, Hunkapiller & Hood, 1981; Kangawa, Minamino, Chino, Sakakibara & Matsuo, 1981; Khachaturian, Watson, Lewis, Coy & Goldstein, 1982; Watson, Akil, Ghazarossian & Goldstein, 1981). Three main [leu]-enkephalin-containing peptides, alpha- and beta- neo-endorphin, dynorphin A (1-8; 1-17) and dynorphin B (1-13; 14-29) are produced from prodynorphin (see review: Akil et al., 1984).

B. Specific Opiate Receptor Subtypes

The existence of multiple receptors was suggested by pharmacological experiments and was further substantiated by biochemical receptor binding studies. Heterogeneity among opiate receptors was initially based on neurophysiological and behavioral observations in dogs with chronic spinal transections (Martin et al., 1976). Three distinct receptor subtypes were identified. The mu receptors preferentially interacted with morphine-like drugs exhibiting responses as meiosis, bradycardia, hypothermia, analgesia and indifference in non-dependent dogs. Kappa receptors selectively responded to

ketocyclazocine and were characterized by the absence of bradycardia, and by a failure to either precipitate or suppress withdrawal in morphine dependent dogs. Further evidence for the existence of kappa receptors was also obtained from smooth muscle preparations. Dynorphin (1-13) and ethylketocyclazocine (EKC) were subsequently found to bind in a selective manner to the kappa receptor and were poorly antagonized by naloxone (Chavkin, James & Goldstein, 1982). In cross-protection studies on guinea pig brain, EKC was more effective than morphine in preventing the inactivation of [^{3}H] EKC binding sites by phenoxybenzamine (Kosterlitz & Leslie, 1978). A third type of receptor identified by Martin and co-workers was the sigma receptor which preferentially responded to N-allylnorphenazocine (SKF-10,047). Sigma receptors were associated with pupillary dilation, tachypnea, tachycardia, induced canine delirium and psychomimetic effects in humans. Sigma receptors are not "true" opioid receptors since they failed to exhibit the stereospecific binding typical of opiate receptors. SKF-10,047 and cyclazocine, unlike morphine, dynorphin and [D-ala^2,D-Leu^5]-enkephalin (DADL), potently displaced phencyclidine binding (Quirion, Hammer, Herkenham & Pert, 1981) suggesting that sigma receptors may actually be phencyclidine (PCP) receptors (Zukin & Zukin, 1981).

Heterogeneity among opioid receptors was further characterized by utilizing bioassay studies. Although the guinea-pig ileum has an abundance of mu and kappa receptors, greater amounts of naloxone were required to reverse the inhibitory effects of kappa agonists (Lord et al., 1977). The mouse vas deferens possesses a mixture of delta and mu receptors, a receptor population which parallel those within the guinea-pig brain (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975). However, unlike the mouse, the vas deferens of the rat contains a population of epsilon receptors that are

quite sensitive to ß-endorphin as compared to morphine and enkephalins. Besides the peripheral system, epsilon receptors are also evident in the central nervous system, originating in the area of the hypothalamus, and innervating a number of brain areas (Schulz et al., 1979). While the agonist, ß-endorphin, binds to the epsilon receptors, it lacks specificity, displaying an affinity for the mu and delta binding sites as well. Morphine, which generally inhibits electrically-evoked muscle contractions, actually potentiated this response in the rat vas deferens (Wuster, Schulz & Herz, 1980). Kosterlitz and his colleagues noted that morphine-like compounds are more potent than enkephalins in inhibiting the electrically-stimulated muscle contractions of isolated guinea-pig ileum. Conversely, met-enkephalin and leu-enkephalin are more potent than morphine in depressing the contractions within the mouse vas deferens, thereby interacting mainly with so-called delta receptors (Lord et al., 1977). Cross-protection studies were consistent with this result showing that dihydromorphine (DHM) protects against inactivation of [^{3}H] DHM binding sites by phenoxybenzamine more effectively than does DADL. Similarly, DADL protects [^{3}H] DADL sites more effectively than does DHM (Robson & Kosterlitz, 1979). However, DADL bound to mu sites with the same effectiveness as delta sites (Chang & Cuatrecasas, 1979).

The mu_1 receptor, binds many, but not all, opiates and enkephalins equally well while mu_2 receptor subtype selectively binds morphine-like compounds more potently than enkephalins (Clark, Houghten & Pasternak, 1988; Pasternak & Wood, 1986). Similarly, delta receptors preferentially binds enkephalins. Naloxazone, an irreversible opiate antagonist, blocked the high affinity of both [^{3}H] DHM and [^{3}H] DADL in saturation studies (Hahn, Carroll-Buatti & Pasternak, 1982). A similar loss of high-affinity binding was obtained with N ethylmaleimide which selectively activates mu_1 sites. In cross

protection studies, both morphine and enkephalin protected [^{3}H] DHM binding from N-ethylmaleimide (Burkhardt, Frederickson & Pasternak, 1982; Nishimura, Recht & Pasternak, 1984; Pasternak, Gintzler, Houghten, Ling, Goodman, Spiegel, Nishimura, Johnson & Recht, 1983; Wolozin & Pasternak, 1981). The presence of distinct high-affinity binding sites suggests that opiate actions are mediated by the different receptor subtypes. Mu$_1$ sites play a role in supraspinal analgesia, a response which may be antagonized by naloxonazine, a selective, irreversible mu$_1$ antagonist (Bodnar, Williams, Lee & Pasternak, 1988). Also, mu$_1$ sites have been implicated in acetylcholine turnover, prolactin release, hypothermia, catalepsy, and some signs of physical dependence (Pasternak & Wood, 1986). On the other hand, mu$_2$ sites are involved in respiratory depression, gastrointestinal transit, bradycardia, growth hormone release, dopamine turnover. The opioid actions of the delta sites were also characterized and have been shown to modulate spinal, as well as supraspinal, analgesia, reversal of endotoxic shock, growth hormone release and dopamine turnover.

The central and peripheral anatomical distribution of opiate receptors may be indicative of functional significance. Autoradiographic techniques facilitated the differentiation among opioid receptor subtypes following labeling with selective ligands. For example, mu and delta receptors are typically labeled with [^{3}H] (Tyr-D-Ala-Gly-MePhe-Gly-ol)-enkephalin (DAMGO) and [^{3}H] D-Pen^2,D-Pen^5-enkephalin (DPDPE), respectively, while kappa receptors are labeled with either [^{3}H] ethylketocyclazocine (EKC) or a more selective ligand, [^{3}H] bremazocine (Atweh & Kuhar, 1977; Goodman & Snyder, 1982; Goodman, Snyder, Kuhar & Young, 1980; Lewis, Khachaturian & Watson, 1985; Lewis, Mishkin, Bragin, Brown, Pert & Pert, 1981; Mansour, Lewis, Khachaturian, Akil & Watson, 1986; Mansour, Khachaturian, Lewis, Akil &

Watson, 1987; Quirion, Weiss & Pert, 1983). Mu binding is densest in areas that are relevant to pain sensation, including the substantia gelatinosa of the spinal cord, the periaqueductal gray, the median raphe nuclei, the dorsomedial thalamus and layer IV of the cerebral cortex. Its involvement is also evident in neural areas of sensory processing, such as superior and inferior colliculi, the lateral and medial geniculate nuclei, amygdala and olfactory bulb. Many of the areas found to be high in delta receptors are parts of the limbic system (e.g., olfactory tubercle, nucleus accumbens and amygdala) that are associated with control of emotions and reward behavior. Dense delta binding was further noted in the cortex and caudate-putamen, sites which are involved in the mediation of sensorimotor responses. Kappa cell bodies were concentrated in sites that mediate sensory processing (e.g., amygdala and olfactory tubercle), neuroendocrine control (e.g., hypothalamus, pituitary gland and median eminence) and regulation of eating and drinking (e.g., nucleus tractus solitaris, parabrachial nucleus, thalamus, medial nuclei of the amygdala and medial hypothalamus) (Lynch, Watt, Krall & Paden, 1985).

Thus, a number of behaviors are modulated by multiple opioid receptors. The following sections will focus on ingestive behavior and the involvement of the different opioid receptors in ingestive behavior.

C. Ingestive Behavior

Ingestive behavior is a complex and integrative process. Information concerning food intake involves the peripheral system, whereby signals are conveyed from areas such as the mouth, stomach, small intestine, liver and pancreas (Carlson, 1986). Within the gastrointestinal tract, regulation is facilitated by hormonal and neural factors (Gonzalez & Deutsch, 1981). However, maintenance of intake is controlled by the central nervous system,

with particular attention given to the hypothalamic PVN, the ventromedial hypothalamus, the lateral hypothalamus, globus pallidus, nucleus accumbens and area postrema (Ritter & Ritter, 1986). Regulation requires the interaction of amines (e.g., dopamine, norepinephrine, epinephrine and serotonin), neuropeptides (e.g., opioid peptides, neuropeptide Y, corticotropin-releasing factor, cholecystokinin, neurotension) and amino acid transmitters (e.g., gamma-aminobutyric acid) (Morley, 1987). The influence of sensory qualities of visual, gustatory and olfactory cues, in conjunction with psychological factors, including taste aversion and appropriate nutrition, also interact with biochemical factors to regulate feeding (Rozin & Schulkin, 1990; Stricker, 1990). Furthermore, the mere presence of such basic nutrients as glucose, amino acids and fatty acids, also determine the occurrence of intake (Carlson, 1986), and subsequently, several theories were proposed to account for their involvement. Changes in the supply of the metabolic fuels to cells appear to be a stimulus for the cessation of hunger rather than depletion of the specific nutrients (Friedman & Stricker, 1976). Glucose is the primary fuel of all cells, and, when glucose levels are low, cells are quickly starved. The glucostatic theory maintains that increases in the rate and utilization of glucose lead to a decrease in hunger and eating. Conversely, decreases in the rates of glucose uptake and utilization excite postulated glucoreceptive cells and thus, result in hunger (Mayer, 1955). Low levels of amino acids also signal the onset of feeding while a cessation occurs when levels returned to normal (Mellinkoff, Frankland, Boyle & Greipel, 1956). The lipostatic theory suggests that eating is controlled by cells that are sensitive to the amounts of lipids in the general circulation. The presence of free fatty acids (Kennedy, 1972) or glycerol (Glick, 1980) may serve as signals for intake. In addition, body temperature has been implicated in feeding (Brobeck, 1948). Food increases the metabolic

activity in cells, and causes an increase in body temperature, although the increase depends upon the type of food consumed. Fats and proteins have the lowest and highest metabolic rates, respectively. The following section will elaborate on opioid peptides and their specific involvement in different forms of ingestive behavior, including free feeding, deprivation-induced feeding, glucoprivic intake, palatable intake and nocturnal feeding.

D. Opioid Peptides and Ingestive Behavior

1. Free Feeding and Deprivation-induced Feeding

Ingestive behavior is regulated by endogenous opioids which typically stimulate feeding. Following the original observation that naloxone, a potent general opioid antagonist, reduces food intake in deprived rats (Holtzman, 1974), further studies demonstrated decrements in short-term food intake in a diversity of species including mice (Brown & Holtzman, 1979), guinea pigs (Schulz, Wuster & Hertz, 1980), woodchucks (Nizielski, Morley, Gosnell, Seal & Levine, 1985), cats (Foster, Morrison, Dean, Hill & Frenk, 1981), sheep (Baile, Keim, Della-Fera & McLaughlin, 1981), wolves (Morley, Levine, Plotka & Seal, 1983b) and humans (Atkinson, 1982), but not raccoons (Nizielski et al., 1985), golden hamsters (Lowy & Yim, 1982) and Chinese hamsters (Billington, Morley, Levine & Gerritsen, 1984). Naloxone exerts a hypophagic response without typically producing aversion or hypoactivity (Lesham, 1984). Naloxone also failed to alter motor activity or operant responding at levels that decrease feeding since the latency to begin consumption and the initial rate of ingestion are unaffected (Kirkham & Blundell, 1984). However, a given bout of consumption terminates earlier than normal following naloxone, suggesting that opiate antagonists affect the maintenance, rather than the initiation of feeding. Naloxone and naltrexone produce their effect at low doses (0.3

mg/kg), but for short (2-4h) durations (Margules, Goldman & Finch, 1979; Sanger, McCarthy & Metcalf, 1981). The suppressive responses of opiate antagonists were demonstrated not only in deprivation situations, but also in non-deprived (Carey, Ross & Enns, 1981; Cooper, 1980; Lowy, Maickel & Yim, 1980; Sanger & McCarthy, 1981) and nocturnal (Brands, Thornhill, Hirst & Gowdey, 1979; Cooper, 1980; Lowy et al., 1980) feeding conditions, as well as hyperphagia induced by 2DG (Lowy et al., 1980; Sewell & Jawaharlal, 1980) and insulin (Rowland & Bartness, 1982) glucoprivation. Naloxone also decreased intake of palatable diets (Apfelbaum & Mandenoff, 1981; Cooper, Barber & Barbour-McMullen, 1985; Cooper, Jackson, Morgan & Carter, 1985) and feeding stimulated by mild tail-pinch stress (Lowy et al., 1980). Further, both naloxone and naltrexone also attenuate intake stimulated by exogenously-administered norepinephrine (Leibowitz & Hor, 1982), muscimol (Morley, Levine & Kneip, 1981), and opiate agonists of mu, kappa, delta and epsilon receptors (Grandison & Guidotti, 1977; Morley & Levine, 1981; Sanger & McCarthy, 1981; Tepperman & Hirst, 1983). Chronic administration of naloxone and naltrexone transiently decreases food intake and body weight in normal non-obese (Mandenoff, Fumeson, Apfelbaum & Margules, 1982; Mann, Pasternak, Hahn, Curreri, Lubin & Bodnar, 1988b; Shimonura, Oku, Glick, Bray, 1982) and genetically obese (Recant, Voyles, Luciano & Pert, 1980) rats.

A reduction of water consumption in fluid deprived animals was also demonstrated following the administration of opiate antagonists (Brown & Holtzman, 1979; Cooper, 1980; Frenk & Rogers, 1979; Holtzman, 1975; Maickel, Braude & Zabik, 1977; Ostrowski, Foley, Lind & Reid, 1980) at doses that do not sustain a conditioned taste aversion (Ostrowski et al., 1980; Wu, Cruz-Morales, Quinan, Stapleton & Reid, 1979) or affect motor responses (Falk, 1971). As observed with food intake, naloxone also does not affect the

rate of water intake, but rather, exerts its effects by shortening the duration of a drinking bout (Reid, 1985; Siviy, Calcagnetti & Reid, 1982). Drinking induced by injection of hypertonic (Brown, Blank & Holtzman, 1980) and hypotonic (Cooper & Gilbert, 1984) saline as well as palatable solutions (Holtzman, 1975; Ostrowski et al., 1980) have also been shown to be antagonized by naloxone.

Beta-chlornaltrexamine (ß-CNA), a non-equilibrium opiate receptor antagonist that alkylates and inactivates opioid receptors (Portoghese, Larson, Jiang, Takemori & Caruso, 1978), produces a long-lasting reduction in daily food intake and body weight gain and attenuates feeding stimulated by selective opioid agonists of the mu, kappa and delta receptors (Gosnell et al., 1987). Further, the trans-3,4-dimethyl-4-phenylpiperidine compounds (Leander, Hart, Lochner, Hynes & Zimmerman, 1982) decrease food intake and body intake weight gain in normal and genetically obese animals (Shaw, Mitch, Leander & Zimmerman, 1990).

In contrast to antagonists, the expectation that agonists would stimulate feeding was initially confirmed by Martin and colleagues in 1963. Morphine-addicted rats significantly increased food intake after daily injection of morphine (Martin, Wikler, Eades & Pescor, 1963). However, morphine in non-dependent animals was later shown to increase feeding as well (Jalowiec, Panksepp, Zolovick, Najam & Herman, 1981). Acute administration of morphine in satiated rats initially depresses, but then stimulates food intake after 4 h (Kumar, Mitchell & Stolerman, 1971). Beta-endorphin (ß-endorphin), an agonist of epsilon, mu and delta receptors, (Schulz, Wuster, Krenes & Herz, 1980) stimulated food intake following administration into the ventromedial hypothalamus (Grandison & Guidotti, 1977), PVN (Leibowitz & Hor, 1982) and lateral ventricles (Baile et al., 1981; McKay, Kennedy, Edens, Williams &

Woods, 1981). Elevated concentrations of ß-endorphin were found in the pituitaries of genetically-obese mice (ob/ob) and rats (Zucker fa/fa) with feeding increasing concomitantly with increases in plasma levels of ß-endorphin (Gibson, Liotta & Krieger, 1981; Govoni & Yang, 1981; Gunion & Peters, 1981; Margules, Moisset, Lewis, Shibuya & Pert, 1978). As compared to their lean littermate controls, these animals exhibited a greater sensitivity to naloxone's suppression of food intake while no such response was obtained from a variety of non-genetic obese animals. Decreased ß-endorphin levels was found in the ventromedial hypothalamus of Zucker rats relative to their lean controls (McLaughlin, Baile & Della-Fera, 1985). Elevated levels of ß-endorphin in the plasma of genetically obese mice were observed at 4 to 6 months of age when obesity already developed, suggesting that increased ß-endorphin may be a consequence rather than the cause of the obesity (Rossier, Rogers, Shibasaki, Guillemin & Bloom, 1979). Interestingly, significant increases were demonstrated in genetically obese mice as early as 4 weeks of age (Recant et al., 1980).

Food deprivation (2-3 days) significantly reduced ß-endorphin in the hypothalamus but not in the pituitary (Gambert, Garthwaite, Pontzer & Hagen, 1980). In contrast, increased ß-endorphin was observed in the striatum which returned to normal levels upon refeeding (Majeed, Larson, Przewlocka, Przewlocki, 1986; Vaswani & Tejwani, 1986). Further, ß-endorphin in satiated rats was increased in the ventromedial hypothalamus, but decreased in the supraoptic nucleus (McLaughlin et al., 1985). In contrast to ß-endorphin's stimulation of food intake, ß-endorphin antibodies in the PVN significantly decreased feeding and drinking (Schulz, Wilhelm & Dirlich, 1984). Central ß-endorphin antibodies also blocked diazepam-induced feeding in rats (Gonzalez, Fernandez-Tome, Sanchez-Franco & Del Rio, 1984). ß-endorphin effects upon

intake may be mediated through the alpha-noradrenergic receptor since phentolamine reduced ß-endorphin-induced feeding (Leibowitz & Hor, 1982).

Ingestive behavior appears to be regulated by the different opioid receptor subtypes (see review: Morley et al., 1983a). Morphine stimulates feeding which is enhanced by repeated peripheral administration (Morley, Levine, Grace & Kneip, 1982a; Thornhill & Saunders, 1983) and administration into the PVN (McLean & Hoebel, 1983) and the ventromedial hypothalamus (Tepperman, Hirst & Gowdey, 1980; Thornhill & Saunders, 1984). The effects were antagonized by naloxone. Whereas ventromedial hypothalamic lesions failed to alter naloxone anorexia (King, Castellanos, Kastin, Berzas, Mauk, Olson & Olson, 1979), PVN lesions significantly attenuated morphine induced feeding (Shor-Posner, Azar, Filart, Tempel & Leibowitz, 1986). In addition, morphine effects upon intake depend upon the internal state of the animal. Morphine increased food and water intake in both freely-feeding and mildly-deprived (4-5 hours) rats, but decreased intake in rats undergoing 24h of deprivation (Kumar et al., 1971; Sanger & McCarthy, 1980, 1981; Jalowiec et al., 1981). The highly selective mu receptor agonist, DAMGO, stimulated food intake after central injection (Gosnell, Levine & Morley, 1986a), whereas the less selective mu agonist, morphiceptin, failed to alter feeding over a wide dose range (Morley, Levine, Gosnell & Billington, 1984).

Dynorphin, which has its best affinity for the kappa receptor (Chavkin et al., 1982), stimulates feeding in satiated rats and mice (Morley & Levine, 1981; Walker, Katz & Akil, 1980). Intracerebroventricular infusion of dynorphin (1-13) elicited eating and grooming but not drinking. Dynorphin (1-17), dynorphin (1-10), dynorphin (1-11) and dynorphin (1-13) as well as the non-opioid dynorphin fragment (6-17) each stimulated intake. Neither dynorphin (1-8) and dynorphin (1-9) were effective. Such kappa sensitive compounds as

ethylketocyclazocine, ketocyclazocine, tifluadom and butorphanol tartrate also stimulated feeding. However, since they are not exclusive kappa agonists, they may exert their effects at other opioid receptor sites. Interestingly, U50,488H and bremazocine, which are highly-selective kappa agonists, are not as effective in stimulating food consumption, suggesting that the other agonists engage other opiate receptor subtypes (Jackson & Cooper, 1986; Lowy & Yim, 1983; Morley, Levine, Grace, Kneip & Zeugner, 1983c; Morley, Levine, Kneip, Grace, Zeugner & Shearman, 1985). Food deprivation decreases dynorphin levels in the hypothalamus and striatum (Vaswani & Tejwani, 1986), as well as the cortex (Majeed et al., 1989; Morley, Elson, Levine & Shafer, 1982b). Microinjections of such kappa agonists as dynorphin and MR2043, into either the PVN or the ventromedial hypothalamus increases feeding (Gosnell, Morley & Levine, 1986b; Scott, Jawaharlal & Hoebel, 1984). Conversely, dynorphin antibodies in the PVN and alpha-neo-endorphin antibodies in the ventromedial hypothalamus significantly inhibit food and water intake (Schulz et al., 1984). Similarly, central dynorphin (1-13) antibodies, but not β-endorphin antibodies elevated the threshold for electrical brain stimulation-induced feeding (Carr, Bak, Gioannini & Simon, 1987). Lesions placed in the globus pallidus and striatum reduced ketocyclazocine-induced feeding without affecting naloxone anorexia (Gosnell, Morley & Levine, 1984a). In contrast, butorphanol tartrate-induced feeding and naloxone anorexia were unaffected by either lesions placed in the hippocampus (Gosnell, Morley, Levine, Kneip, Frick & Elde, 1984b), pineal gland, optic nerve or superior cervical ganglion (Gosnell, Waggoner, Morley & Levine, 1985a) or by parasagittal medial hypothalamic knife cuts (Gosnell, Romos, Morley & Levine, 1985b).

Delta opioid receptor agonists also stimulate feeding, including DADL in the ventromedial hypothalamus (Tepperman & Hirst, 1983) and [D-Ala^2-Met^5]-

enkephalin (DAME) in the PVN, perifornical hypothalamus and amygdala, but not the septum, hippocampus, globus pallidus, periaqueductal grey, midbrain tegmentum and fourth ventricle (McLean & Hoebel, 1983; Stanley, Lanthier & Leibowitz, 1989). In addition, the specific delta agonists, DPDPE and [D-Ser^2, Leu^5]-enkephalin-Thr^6 (DSLET), each enhanced consumption after intracerebroventricular administration (Gosnell et al., 1986a; Stanley et al., 1989).

The sigma receptor has also been implicated in ingestive behavior, although its opioid action has been questioned (Zukin & Zukin, 1981). N-allylnorphenazocine (SKF-10,047) stimulates food intake which was reversed by naloxone. Moreover, the feeding induced by ketocyclazocine but not morphine, is potentiated by repeated pretreatment with SKF-10,047 (Gosnell, Morley & Levine, 1983). Finally, high doses of SKF-10,047 in the PVN increased feeding (Scott et al., 1984).

Essentially, agonist studies indicate that food intake is mediated by interactions among mu, kappa, delta, and to a lesser extent, epsilon and sigma receptors. To investigate the involvement of specific opiate receptor subtypes in ingestion, opiate antagonists need to be employed. The general non-specific opiate antagonists, naloxone and naltrexone, have yielded consistent results under a variety of situations, including spontaneous intake (Carey et al., 1981; Cooper, 1980; Lowy et al., 1980; Sanger & McCarthy, 1981), deprivation intake (Brands et al., 1979; Brown & Holtzman, 1979; Holtzman, 1974), 2DG glucoprivation (Lowy et al., 1980; Sewell & Jawaharlal, 1980) insulin glucoprivation (Rowland & Bartness, 1982), intake of palatable diets (Apfelbaum & Mandenoff, 1981; Cooper et al., 1985) and nocturnal feeding (Brands et al., 1979; Cooper, 1980; Lowy et al., 1980). ß-CNA, a nitrogen mustard derivative of naltrexone (Portoghese et al., 1978), is a non-equilibrium

opioid receptor antagonist shown to inhibit the action of opioid agonists on the guinea-pig ileum (Takemori et al., 1981) and mouse vas deferens (Ward, Portoghese & Takemori, 1982a). ß-CNA alkylates opioid receptors and, unlike naloxone and naltrexone, causes a long-term inactivation of the receptors. Intracerebroventricular administration of ß-CNA produces long-lasting (2-4 days) decreases of morphine-induced analgesia (Portoghese et., 1978; Larson & Armstrong, 1980) and reductions in daily food intake and body weight gain (Gosnell et al., 1987). In addition, ß-CNA attenuates feeding induced by selective agonists of mu (DAMGO), kappa (dynorphin) and delta (DSLET) receptors. The trans-3,4-dimethyl-4-phenylpiperidine compounds (Leander et al., 1982) bind to opiate receptors, but with a longer duration of action than naloxone. These opioid antagonists (e.g., LY117413, LY88329, LY99335) significantly decrease food consumption and body weight gain in normal non-obese and genetically obese animals (Shaw et al., 1990).

The general opioid antagonists are important pharmacological tools in assessing the role of the opioid receptors in appetite control. However, because of their lack of specificity, differentiating the role of the specific receptor subtypes under a variety of conditions in ingestive behavior is rather difficult. Subsequently, this led to the development of highly-selective antagonists. ß-FNA, the fumarate methyl ester derivative of naltrexone (Portoghese et al., 1980) is a highly-selective, non-equilibrium mu antagonist and a reversible, shorting-acting kappa agonist (Takemori et al., 1981). ß-FNA displays short-acting and naloxone-reversible antinociception, presumably exerting its effects at the kappa receptor (Ward, Portoghese & Takemori, 1982b). In contrast, ß-FNA exhibited a long-lasting antagonistic action at the mu receptor. Further investigations indicated that ß-FNA responded similarly in ingestive behavior. Intracerebroventricular administration of ß-FNA stimulates

food intake at 2 hours after injection, but reduces food and water intake for up to 72 h in non-deprived rats without altering motor activity (Ukai & Holtzman, 1988). β-FNA injected into the PVN also attenuated deprivation-induced feeding (Levine et al., 1989) and decreased DAMGO hyperphagia (Levine, Grace & Billington, 1991). The mu1 high affinity binding site for opiates and enkephalins has been characterized utilizing naloxonazine, a selective, long-acting mu1 antagonist (Pasternak & Wood, 1986). Pretreatment (24h) with naloxonazine decreases free feeding, deprivation-induced feeding and morphine-induced hyperphagia, but fails to alter feeding induced by 2DG, the kappa agonists, ethylketocyclazocine and dynorphin, or the delta agonist DADL (Mann, Arjune, Romero, Pasternak, Hahn & Bodnar, 1988a; Simone, Bodnar, Goldman & Pasternak, 1985). Furthermore, naloxonazine attenuates food consumption and body weight gain in adolescent and adult rats following chronic administration over 14 days (Mann et al., 1988b).

Nor-BNI displays high affinity for the kappa receptors in smooth muscle preparations (Portoghese et al., 1987) and *in vivo* antinociceptive assays, whereby it significantly but reversibly antagonizes the analgesic responses of the kappa agonists, ethylketocyclazocine and U50,488H (Takemori et al., 1988). Nor-BNI antagonizes the anticonvulsant actions of U50,488H, but not of DAMGO, a mu agonist (Tortella, Echevarria, Lipkowski, Takemori, Portoghese & Holaday, 1989). Kappa antagonists have also been implicated in the regulation of food consumption. Nor-BNI inhibits feeding induced by electrical stimulation of the lateral hypothalamus (Carr, Bak, Simon & Portoghese, 1989). Whereas central Nor-BNI marginally decreased feeding following food deprivation, it potently antagonized intake stimulated by mu (DAMGO) and delta (DSLET) receptor agonists (Levine et al., 1990). A weaker kappa receptor antagonist,

MR2266 (Smith, 1987; Valentino, Katz, Medzihrasky & Woods, 1983) also attenuates hyperphagia in rats deprived of food (Sanger, McCarthy, Lord & Smith, 1983).

The enkephalin analog, ICI174,864 (Cotton, Giles, Miller, Shaw & Timms, 1984) stimulates the mouse vas deferens by potently and selectively binding to the delta receptor. As a short-acting antagonist, it quickly degrades into a peptide exhibiting affinity for the mu receptor (Cohen, Shuman, Osborne & Gesellchen, 1986). Central administration of ICI174,864 reduces nocturnal intake (Jackson & Sewell, 1985a) and blocks DADL hyperphagia (Jackson & Sewell, 1985b). However, ICI174,864 administration also produces motor impairment (Long, Petras & Holaday, 1988) which confounds its hypophagic properties. A synthetic peptide analog of leu-enkephalin [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE), displays high affinity for delta receptors, moderate affinity for mu receptors and negligible affinity for kappa receptors (Bowen et al., 1987). DALCE binds covalently to delta receptors by forming a disulfide bond with a sulfhydryl group in the binding site, and subsequently, readily dissociates from the mu receptors but not the delta receptors. Essentially then, DALCE acts as short-acting agonist at the delta receptor and secondarily at the mu receptor. DALCE increases hot-plate latencies that was antagonized by naloxone and the delta antagonist, M80 (Calcagnetti, Fanselow, Helmstetter & Bowen, 1989a). On the other hand, DALCE subsequently produces a selective, long-lasting antagonism at the delta receptor. Pretreatment (24h) with DALCE selectively blocks analgesia produced by the delta-selective agonist, DPDPE, but not by mu (DAMGO) or the kappa (U50,488H) agonists on the formalin test. Basal nociception is unaltered by intraventricular administration of DALCE (Calcagnetti et al., 1989a; Calcagnetti, Bowen & Holtzman, 1989b; Jiang, Bowen, Mosberg, Rothman &

Porreca, 1990).

It is important to recognize that while the antagonists display an affinity for specific receptors, some antagonists exhibit greater selectivity than others. For example, Nor-BNI binds to the kappa receptor, relative to the mu receptor, with a 170-fold greater affinity. Similarly, β-FNA reversibly binds to the kappa receptor for a duration of 6 hours, while irreversibly binds to mu receptor for a period of 72 hours. This is in contrast to DALCE, which displays some affinity for the mu receptor while exhibiting a greater selectivity for the delta receptor. At relatively low doses, Nor-BNI and β-FNA, but not DALCE, may exert a dose response effect indicative of their greater selectivity. While selectivity is evident by a drug's action, it should be kept in mind that such response may not be absolute. Therefore, while antagonists display an affinity for specific receptors, interpretation should be approached cautiously.

II. Glucoprivic Feeding and Opioid Receptor Subtype Antagonists

A decrease in glucose utilization is associated with an increase in food consumption, and conversely, a return of blood glucose to normal levels is typically accompanied by cessation of hunger (Mayer, 1955). Glucoprivation may be induced by the antimetabolic analogue, 2-deoxy-D-glucose (2DG), which competes with intracellular glucose, and insulin, which promotes the uptake of glucose into non-neural tissues thus resulting in hypoglycemia (Brown, 1962). However, 2DG- and insulin- induced feeding are dissociated based on their mechanism of action. Lesions placed in the ventromedial hypothalamus, medial forebrain bundle, midbrain tegmentum, zona incerta and sub-diaphragmatic vagotomy attenuate 2DG, but not insulin-induced feeding (Booth, 1972; Grossman & Grossman, 1977; McDermott, Alheid, Kelly, Halaris & Grossman, 1977; Rowland & Engle, 1978; Sclafani, Gale & Springer, 1975;

Walsh & Grossman, 1975). In contrast, bilateral adrenal demedullation reduces insulin, but not 2DG hyperphagia (Booth, 1972; Grossman, Cummins & Ivy, 1947).

Glucoprivation is mediated by the endogenous opioid system (see review: Morley et al., 1983a). Naloxone and naltrexone decrease 2DG hyperphagia with greater effectiveness than insulin-induced feeding (Lowy et al., 1980; Ostrowski et al., 1981; Yim, Lowy, Davis, Lamb & Malven, 1982). These opioid antagonists either failed to alter (Lowy et al., 1980) or marginally reduced feeding induced by insulin (Levine & Morley, 1981; Ostrowski et al., 1981; Rowland & Bartness, 1982). The delta antagonist, ICI174,864, also failed to affect 2DG hyperphagia (Jackson & Sewell, 1985b).

III. Palatability and Opioid Receptor Subtype Antagonists

Highly palatable diets, introduced as a model of obesity, result in hyperphagia and body weight gain (Sclafani, 1978). The endogenous opioid system has been implicated in the control of such diets. The kappa agonists, U50,488H, tifluadom, ethylketocyclazocine and bremazocine, stimulate the intake of diets consisting of mixture of powered standard rat food, sweetened condensed milk and water (Jackson & Cooper, 1985). When injected into the lateral ventricle, morphine facilitated the intake of sweetened milk (Belluzzi & Stein, 1982). Opioids also enhanced the taste of sweet and nonsweet solutions. Morphine injection stimulates the consumption of not only saccharin and sucrose (Calcagnetti & Reid, 1983; Cooper, 1983; Czirr & Reid, 1986), but also of isotonic (0.9%) (Bertino, Abelson, Marglin, Neuman, Burkhardt & Reid, 1988) and hypertonic (3.0%) (Kuta, Bryant, Zabik & Yim, 1984) saline. Moreover, central administration of the mu agonist, DAMGO, and the delta agonists, [D-Thr^2,Leu^5]enkephalin-Thr^6 (DTLET) and DPDPE, significantly increase the intake

of hypertonic (0.6%) saline solutions (Gosnell, Majchrzak & Krahn, 1990; Gosnell & Majchrzak, 1990). In addition to increasing the preference for sweet and salty solutions, opioids also alter the selection of macronutrients. Morphine facilitates fat intake while decreasing carbohydrate consumption on self-selection diets (Marks-Kaufman & Kanarek, 1980). In contrast, morphine increases protein intake following food deprivation (Shor-Posner et al., 1986).

Opiate antagonists further demonstrate the involvement of opioids in mediating palatable consumption. Naloxone and naltrexone attenuate the preference and intake of highly palatable diets and solutions (Apfelbaum & Mandenoff, 1981; Cooper & Gilbert, 1984; Cooper et al., 1985a,b; Marks-Kaufman & Kanarek, 1981). The irreversible mu opioid receptor antagonist, β-FNA, reduces the intake of a high-fat diet while the mu1 antagonist, naloxonazine, slightly stimulates food intake. The selective delta antagonist, ICI174,864, also attenuates intake of palatable diets, however, its response is associated with motor dysfunctions (Islam & Bodnar, 1990). Further, the putative kappa antagonist, MR2266, was shown to decrease sweetened milk consumption (Cooper et al., 1985a) and saccharin and glucose solutions (Calcagnetti, Calcagnetti & Fanselow, 1990). Similarly, intracerebroventricular administration of Nor-BNI suppresses intake of the palatable solutions (Calcagnetti et al., 1990).

IV. Nocturnal Intake

Rats ingest food during discrete episodes separated by intervals of non-eating (Le Magnen, 1981). Larger meals and shorter between-meal intervals are evident during the animal's active cycle, namely at night. Thus, during the day rats eat small, infrequent meals. Endogenous opioids appear to vary according to the circadian rhythm. Met-enkephalin levels increase in the

hypothalamus, PVN and VMH during the dark phase when most food is ingested (McLaughlin, Baile & Della-Fera, 1987). Elevated levels of dynorphin have also been observed in the hypothalamus during the active feeding cycle of the rat (Przewlocki, Larson, Konecka, Gramsch, Herz & Reid, 1983). The opiate antagonists, naloxone and naltrexone, were shown to reduce nocturnal feeding (Brands et al., 1979; Cooper, 1980; Jalowiec et al., 1981; Mandenoff, Bertiere, Betoulle & Apfelbaum, 1984).

E) Rationale

Characterization of opiate receptors utilizing biochemical, bioassay, pharmacological and behavioral techniques demonstrated the existence of several distinct receptor subtypes, namely mu, kappa, delta, sigma and epsilon receptors (Lord et al., 1977; Martin et al., 1976; Schulz et al., 1979). Two pharmacologically distinct binding sites of the mu receptor, the high-affinity mu1 binding site and the low-affinity morphine selective mu2 binding site, were identified (Wolozin & Pasternak, 1981). The mu1 receptor has been implicated in supraspinal analgesia, hypothermia, prolactin release, catalepsy, free-feeding and deprivation-induced feeding (Pasternak & Wood, 1986; Simone et al., 1985). On the other hand, mu2 binding site mediates gastrointestinal tract transit, respiratory depression, growth hormone release, dopamine turnover (Pasternak & Wood, 1986), glucoprivic feeding (Simone et al., 1985) and intake of palatable diets (Islam & Bodnar, 1990). The kappa opiate receptor subtype is involved in spinal analgesia and sedation (Martin et al., 1976), whereas the delta receptor modulates both spinal and supraspinal analgesia, growth hormone release, dopamine synthesis and reversal of endotoxic shock (Lord et al., 1977). The sigma and the epsilon receptors are involved in the mediation of a number of behaviors, including ingestion. However, the sigma

receptors are not true opiate receptors (Zukin & Zukin, 1981) and the epsilon receptors, although displaying stereospecific binding, are still poorly characterized (Schulz et al., 1979). As a result, these two receptors will not be evaluated in ingestion. In addition, the lack of specific antagonists for these receptors also hinder their consideration in ingestive behavior.

Agonists for the opiate receptor subtypes facilitate the ingestion of food. Morphine stimulates feeding following both peripheral (Thornhill & Saunders, 1983) and central (McLean & Hoebel, 1983; Tepperman et al., 1981) injection. However, morphine exhibits differential responses depending on the condition of the animal. Following the administration of morphine, food intake increases in both non-deprived and mildly-deprived rats, while morphine suppresses feeding in more prolonged food deprivation (Jalowiec et al., 1981; Sanger & McCarthy, 1980, 1981). The enkephalin analog, DAMGO, which displays high affinity for mu receptors, also potently stimulates feeding in non-deprived rats (Gosnell et al., 1986b). Dynorphin and such other kappa agonists as U50,488H, bremazocine and ethylketocyclazocine, enhance consumption following systemic and central injection (Jackson & Cooper, 1986; Lowy & Yim, 1983; Morley et al., 1983a). Hyperphagic responses are also observed with delta receptor agonists, DADL, DSLET and DPDPE, and the epsilon receptor agonist, ß-endorphin (Gosnell et al., 1986b; Grandison & Guidotti, 1977; Tepperman & Hirst, 1983).

The opiate antagonists, naloxone and naltrexone, attenuate feeding in a variety of situations, including spontaneous feeding (Carey et al., 1981; Cooper, 1980; Lowy et al., 1980; Sanger & McCarthy, 1981), deprivation feeding (Brands et al., 1979; Brown & Holtzman, 1979; Holtzman, 1974), 2DG glucoprivation (Lowy et al., 1980; Sewell & Jawaharlal, 1980), intake of palatable diets (Apfelbaum & Mandenoff, 1981; Cooper et al., 1985) and

nocturnal intake (Brands et al., 1979; Cooper, 1980; Jalowiec et al., 1981). Naloxone also blocks the feeding response induced by opiate receptor subtype agonists, including morphine, dynorphin, ethylketocyclazocine and ß-endorphin (Grandison & Guidotti, 1977; Morley & Levine, 1981; Sanger & McCarthy, 1981). ß-chlornaltrexamine, which alkylates all opiate receptors, suppresses free feeding, body weight gain, and feeding induced by DAMGO, dynorphin and DSLET (Gosnell et al., 1987). A similar response was obtained with the phenylpiperidine compounds (Shaw et al., 1990). While these antagonists are important for characterizing the opiate receptors' response in ingestive behavior, they nonetheless, lack the ability to distinguish among the specific opiate receptor subtype, and as a result, unable to differentiate the different forms of feeding.

Naloxonazine, a mu1 antagonist, significantly reduces food intake under non-deprived, deprived, and morphine-induced feeding condition. However, pretreatment with naloxonazine failed to alter hyperphagia induced by 2DG, dynorphin, EKC or DADL indicating that these responses are mediated at sites unrelated to mu1 (Mann et al., 1988a; Simone et al., 1985). Chronic treatment of over 14 days with naloxonazine attenuates food intake and body weight gain in adult and adolescent rats (Mann et al., 1988b). ß-FNA, acting as an agonist at the kappa receptor, facilitates food intake in freely feeding animals for up to 2h after central injection. ß-FNA subsequently alkylates mu receptors and reduces food and water intake for up to 72h (Ukai & Holtzman, 1988). A suppressive response of deprivation-induced feeding was demonstrated with ß-FNA injection into the PVN (Levine et al., 1989). This antagonist also reduces the feeding induced by DAMGO, but only transiently affecting DSLET- and U50,488H- induced feeding (Levine et al., 1991). ß-FNA, but not naloxonazine, blocked intake of a high-fat diet intake suggesting mu2 opioid receptor

subtype mediation (Islam & Bodnar, 1990).

The first series of experiments evaluated the central actions of β-FNA since opioid actions on ingestive behavior are centrally mediated (Morley et al., 1983a). β-FNA is investigated under several conditions to determine its effect in mediating ingestive behavior. Its short-term, reversible kappa agonist effect and long-term, irreversible mu antagonist actions were examined in male rats under free feeding conditions, whereas its antagonist effects were examined under food deprivation and glucoprivic conditions. Changes in food intake following intracerebroventricular (ICV) administration of β-FNA were thus evaluated in a) short-term (3-6h) and long-term (24-72h) free feeding conditions, b) a long-term (24-72h) pretreatment following 24h of food deprivation, c) long-term (24h) pretreatment upon 2DG hyperphagia and d) short-term (0.5-3h) responses after pretreatment with Nor-BNI or β-FNA. If β-FNA stimulates short-term feeding and if its effects are blocked by Nor-BNI, it would suggest a kappa opioid receptor mediation. In contrast, if β-FNA inhibits long-term responses induced by free-feeding, deprivation-induced feeding and glucoprivic feeding, the mu opioid receptor subtype is responsible.

The second set of experiments evaluated central pretreatment of Nor-BNI, a short-term kappa antagonist, on nocturnal, glucoprivic and palatable intakes in male rats. Nor-BNI suppresses feeding induced by food deprivation (Levine et al., 1990) as well as feeding induced by electrical stimulation of the lateral hypothalamus (Carr et al., 1989). This short acting antagonist also blocked the hyperphagic response of DAMGO, U50,488H and DSLET suggesting that kappa mediation may be involved in both mu and delta opioid actions (Levine et al., 1990). The kappa receptor has been implicated in nocturnal feeding as evidenced by increase levels of dynorphin in the hypothalamus during the dark phase of the circadian rhythm (Przewlocki et al., 1983).

Naloxone and naltrexone were shown to decrease nighttime intake (Brands et al., 1979; Cooper, 1980; Jalowiec et al., 1981; Mandenoff et al., 1984). Both opiate antagonists also were more effective in decreasing the hyperphagia of 2DG rather than of insulin (Lowy et al., 1980; Ostrowski et al., 1981). 2DG feeding, while decreased by naloxone, was unaffected by naloxonazine and ICI174,864 (Jackson & Sewell, 1985b; Lowy et al., 1980; Simone et al., 1985). Thus, Nor-BNI effects on nocturnal intake and glucoprivic intake, following the administration of 2DG, are evaluated to determine the influence of kappa receptor responses in this form of feeding.

The kappa receptor subtype plays a role in the mediation of palatable diets (Sclafani, 1978). Specific and partial agonists, including U50,488H, bremazocine, ethylketocyclazocine and tifluadom stimulate the intake of a wet mash diet (Jackson & Cooper, 1985) which were reversed by naloxone (Cooper et al., 1985). In contrast, Nor-BNI suppresses intake of palatable solutions following intracerebroventricular administration (Calcagnetti et al., 1990). Therefore, Nor-BNI is employed to provide further support for kappa receptor involvement on palatable intake.

The third series of experiments examined the role of the delta receptor in mediating ingestive behavior. Twenty-four hour pretreatment with DALCE antagonizes the analgesia produced by DPDPE, but not DAMGO or U50,488H, indicating delta receptor mediation (Calcagnetti et al., 1989a). The short-term agonistic and long-term antagonistic action of DALCE were evaluated under various forms of feeding behavior including a) short-term (2-10h) and long-term (24-96h) effects on free feeding, b) long-term (24-96h) responses following food deprivation, c) long-term (24h) pretreatment of DALCE upon 2DG glucoprivation, d) long-term DALCE pretreatment upon exposure to a palatable diet and e) short-term (2-10h) effects following pretreatment of opiate

antagonists, naltrexone, Nor-BNI, ß-FNA and long-term DALCE. If DALCE enhanced short-term food intake and if such a response is blocked by long-term DALCE pretreatment, it would indicate a delta mediation. A similar argument may be made with long-term DALCE pretreatment effects upon free feeding, food deprivation, glucoprivation and palatability.

ß-FNA and DALCE were evaluated under similar situations with the exception of the high-fat diet intake condition, which was evaluated earlier in our laboratory using ß-FNA (Islam & Bodnar, 1990). Nor-BNI's response was not examined upon deprivation-induced feeding as Levine and co-workers showed that Nor-BNI only minimally altered food intake (Levine et al., 1990). Nocturnal intake was assessed using Nor-BNI because its shorter duration of action, and as a result, its hypophagic response was evaluated at a time when intake was evident. Moreover, ß-FNA and DALCE also produced a measurement of nocturnal feeding as intake was determined at 12h (which is at the end of the light cycle) following central injection and also determined 24h after injection. These specific antagonists were utilized in different feeding conditions to allow a careful examination of the interaction of the different receptor subtypes in ingestion. However, it should be kept in mind that while the three antagonists, ß-FNA, Nor-BNI and DALCE, selectively binds to the mu, kappa and delta receptors, respectively, selectively is not absolute. ß-FNA and Nor-BNI display a greater selectively for their receptors relative to DALCE and also exert responses at relatively lower doses as compared to DALCE.

A within group design was initially attempted using equal number of animals in each situations. However, since attrition of animals occurred because of illness or displaced cannula, a more conservative method of between subjects design was employed.

General Methods

Subjects:

Adult male albino Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA., 350-500g) were used in all experiments. The animals were housed individually in wire mesh cages and maintained on a 12h light (7:00 AM) : 12h dark (7:00 PM) cycle, with Purina Rat Chow and tap water available ad libitum, except where specifically stated in the protocol. The ambient temperature of the experimental room varied between 22 C and 25 C.

Lateral Ventricular Cannulations:

In preparation for intracerebroventricular (ICV) cannulations, each animal was treated initially with chlorpromazine hydrochloride (3 mg/ml normal saline/kg body weight, intraperitoneally (i.p.)), and then anesthetized 15 minutes later by ketamine hydrochloride (100 mg/ml normal saline/kg body weight, intramuscularly, (i.m.)). Animals were considered anesthetized when they failed to display corneal and pinna reflexes and when no paw withdrawal following pinching was demonstrated.

One stainless steel 22 gauge guide cannula (Plastics Products) was stereotaxically (Kopf Instruments) implanted so that its tip was positioned 0.3 mm above the left lateral ventricle. With the incisor bar set at +5 mm, coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture, 3.6 mm from the top of the skull. The cannula was secured to three stainless steel anchor screws with dental acrylic. A one week recovery period was provided before behavioral testing began to allow full clearance of the drug following surgery.

Before and after surgery, baseline food intakes and body weights were

determined over three consecutive days. Only animals with normal food intake, between 17 and 28 g, and body weight gain (3-5 g/day), were used in the experiments. Following completion of testing, each cannulated rat was sacrificed with an overdose of Euthanasia (No. 5, H. Schein Co.), a barbiturate mixture. Intracerebroventricular cannula placements were then verified by visual inspection. All animals in the experimental procedures met the criteria for localization in the lateral ventricle.

Injection and Drugs:

All intracerebroventricular injections were in 10μl volumes of 0.9% normal saline, which were administered at a rate of 1μl every 10 sec through a stainless steel internal cannula (28 gauge, Plastics Products). The cannula protruded 0.5 mm past the tip of the guide cannula that was connected to a Hamilton microsyringe by polyethylene tubing.

ß-FNA and Nor-BNI were purchased from Research Biochemicals, INC., and were dissolved in distilled water. Between conditions, the solutions were stored at 4 C for a maximum of two weeks. Naltrexone (NTX), which was dissolved in saline, and 2-deoxy-D-glucose, which was dissolved in distilled water, were purchased from Sigma Chemical Company. DALCE was synthesized by Peninsula Laboratories by solid phase techniques. Purification of the drug was by thin-layer chromatography and the purity was checked by reverse phase high-performance liquid chromatography. DALCE was prepared on the day of injection and was dissolved in 0.2M hydrochloric acid. The pH was then raised to 8.0-8.5 by adding 0.2M sodium hydroxide.

High Fat Diet:

The high-fat diet consists of 67% ground laboratory chow and 33%

vegetable shortening (Crisco), with a total of 5.5 Kcal/gram and 11.3% protein, 61.3% fat and 27.4% carbohydrate. Fresh servings of the diet were presented on each occasion. Injection and non-injection conditions occurred at 120 hour intervals to determine whether rats returned to baseline levels. High-fat diet was served fresh for a period of 2 hours, with measurement of intake at 1 and 2 hours following each injection. Spillage was adjusted for by placement of paper under the wire cage whereby the food particles were collected and measured.

Statistical Analysis:

Analysis of variance were performed to assess significant effects among treatments and conditions. Dunnett comparisons were used to determine experimental differences from control means whenever appropriate. Paired t-tests comparing the vehicle and a given drug dose condition were used to evaluate significant effects, as in Experiment 1B. Since a mixed design was employed, the more conservative between-subjects ANOVA was used on which within-subject variance was not partialled out and was included in error terms.

Experiment 1: Beta-funaltrexamine and Ingestive Behavior

1A. Free Feeding Protocol

Microinjections were administered at the beginning of the light cycle since prior to the onset of the light cycle, a large bout of feeding occurs (Le Magnen, 1981). Food intake was measured at 3, 6,12, 24, 48 and 72h after each injection. A total of 4 microinjection conditions were administered at weekly intervals to allow for full drug clearance and the recovery of body weight, and to prevent carry over effects. Rats received subsets of the following intracerebroventricular treatments of ß-FNA: (a) vehicle (n=15), (b) 1µg (n=6), (c) 5µg (n=5), (d) 10µg (n=14) and (e) 20µg (n=9). ß-FNA will reversibly bind to the kappa binding sites on a short-term basis and irreversibly bind to the mu binding sites on a long-term basis (Ward et al., 1982a,b). Drug doses and testing intervals were chosen for their ability to exert peak effects upon mu and kappa binding sites (Ukai & Holtzman, 1988). Food intake, in this and subsequent experiments, was measured by weighing food pellets prior to and after each experimental conditions, including adjustment for spillage, which was measured by placing a paper towel under each wire mesh cage and collecting and weighing the food particles. An assessment of body weight was made prior to, and at 24, 48 and 72 hours following each injection.

1B. Food Deprivation Protocol

All injections occurred between 1 and 2 hours into the light cycle. Between two and three microinjection conditions were administered at weekly intervals to allow for full drug clearance and recovery of body weight. Cannulated animals received the following ß-FNA injection: (a) vehicle (n=15), (b) 10µg (n=5) and (c) 20µg (n=9). ß-FNA was administered 24h prior to the

measurement of food intake to allow for the development of irreversible binding to the mu binding site (Ward et al., 1982a,b). Just prior to each injection all rats were deprived of food, but allowed access to tap water ad libitum. Food was reintroduced twenty-four hours later and intake was measured at 2, 4, 24 and 48h following injection. Body weight was measured prior to and at 24, 48 and 72h after injection.

1C. Glucoprivic Feeding Protocol

Injections were administered between 1 and 2h into the light cycle. Cannulated animals received a maximum of four of the following conditions in which ß-FNA was administered intracerebroventricularly and 2DG administered intraperitoneally at weekly intervals: (a) vehicle (10µl, ICV)-vehicle (1.5 ml normal saline/kg body weight, IP) (n=12), (b) vehicle-2DG (650mg/kg, IP) (n=12), (c) ß-FNA (10µg)-2DG (n=7), (d) ß-FNA (20µg)-2DG (n=12). ß-FNA was administered 24h prior to 2DG to allow for the development of irreversible binding to the mu binding site. The presence of peristaltic-like movements immediately following the administration of 2DG was indicative of an adequate injection. Food intake was measured at 2, 4, 6, and 24 hours after systemic treatment. Body weight was determined immediately prior to, and at 24 and 48 hours following each injection condition.

1D. ß-FNA/Nor-BNI Protocol

A maximum of 5 microinjection conditions were administered at the beginning of the light cycle at weekly intervals: (a) vehicle (10µl, ICV)-vehicle (10µl, ICV) (n=12), (b) vehicle-ß-FNA (10µg, ICV) (n=12), (c) Nor-BNI (10µg, ICV)-ß-FNA (10µg, ICV) (n=12), (d) Nor-BNI (10µg, ICV)-vehicle (n=12) and (e) ß-FNA (10µg, ICV) ß-FNA (10µg, ICV) (n=9). For the first four conditions, one hour

elapsed between the first and second intracerebroventricular microinjections since Nor-BNI reversibly binds to kappa binding sites (Portoghese et al., 1987). For the fifth condition, a period of twenty-four hours elapsed between microinjections to allow β-FNA antagonism binding of the mu binding sites. Measurement of food intake was determined at 0.5, 1, 2 and 3h following the second microinjection.

Results

1A. Free Feeding

Figure 1 indicates the effect of different doses of β-FNA upon free feeding for the first 12h (upper portion) and for 24-72h (lower panel) after injection. As indicated by the stars in the upper panel, all β-FNA doses significantly increased food intake 3h ($F_{4,44}=12.16$, $P<0.0001$) and 6h ($F=7.41$, $p<0.0001$), but not 12h ($F=0.83$) following microinjection. A dose dependent decrease in free feeding was observed at 24h ($F=6.37$, $P<0.0004$), 48h ($F=7.08$, $P<0.002$) and 72h ($F=2.87$, $P<0.034$) after injection (noted by the stars on the lower panel). In order words, the lower doses of β-FNA, 1μg and 5μg, failed to alter long-term food intake while the higher doses, 10 and 20 μg, did. The 10μg dose of β-FNA produced a smaller degree of inhibition than the 20μg dose at 24h (35% vs. 41%), 48h (25% vs. 36%) and 72h (7% vs. 20%) after injection. The response was time-dependent as observed by the degree of inhibition which grew progressively smaller over subsequent 24-hour periods between 24 and 72h after the injection.

Table 1 summarizes the significant differences in body weight during free feeding among vehicle and β-FNA dose conditions ($F_{4,44}=9.38$, $P<0.0001$) and across the injection time course ($F_{2,88}=52.70$, $P<0.0001$). Whereas vehicle

and the lower (1-5 μg) doses of β-FNA failed to alter body weight across the time course, the higher (10-20 μg) doses of β-FNA significantly decreased body weight (indicated by the asterisks). The time course and pattern of body weight suppression by the two higher doses of β-FNA paralleled the inhibitory response demonstrated for food intake. The 20μg β-FNA dose, relative to the lower 10μg dose, displayed greater reductions in body weight which grew progressively smaller over time. An assessment of the ratio of body weight to food intake was evaluated across all β-FNA doses at 24, 48 and 72h after injection to determine whether β-FNA was altering feeding efficiency. Significant differences of this particular ratio failed to occur among conditions ($F_{4,44}=1.83$), across times ($F_{2,88}=1.04$) or for the interaction between conditions and times.

1B. Deprivation-induced Feeding

Figure 2 illustrates the effect of different doses of β-FNA upon deprivation-induced feeding for the first 4 hours (upper panel) and for 24-48 hours (lower panel) following the reintroduction of food. As indicated by the stars in the upper portion of the figure, both doses of β-FNA significantly reduced food intake at 2h (10μg: $t_{10}=2.38$, $P<0.05$; 20μg: $t_{13}=3.96$, $P<0.01$) and 4h (10μg: $t=2.52$, $P<0.05$; 20μg: $t=4.06$, $P<0.01$) following the reintroduction of food. β-FNA suppressed the short-term compensatory increase of deprivation-induced feeding with the 10μg dose significantly decreasing feeding by 33% at 2h and by 30% at 4h, whereas the 20μg dose significantly suppressed intake by 49% at 2h and 43% at 4h. The lower panel indicates a dose dependent decrease in deprivation-induced feeding at 24h (10μg: $t=3.51$, $P<0.02$; 20μg: $t=3.24$, $P<0.02$) and 48h (20μg: $t=5.59$, $P<0.01$) following food reintroduction. The 10μg β-FNA dose significantly reduced food

Figure 1. Alterations in spontaneous food intake (g) following central administration of vehicle or beta-funaltrexamine (β-FNA) over a 72h time course. Intake over the first 3-12h is indicated in the upper panel and intake over 24-72h is indicated in the lower panel. The stars indicate significant differences in food intake following given doses of β-FNA compared to vehicle at each corresponding time point (Dunnett comparisons, $P<0.05$). Standard errors of the mean (S.E.M.) across conditions at 3h (0.3-0.5), 6h (0.4-0.5), 12h (0.5-1.2), 24h (1.0-3.0), 48h (0.9-2.6) and 72h (0.7-2.2).

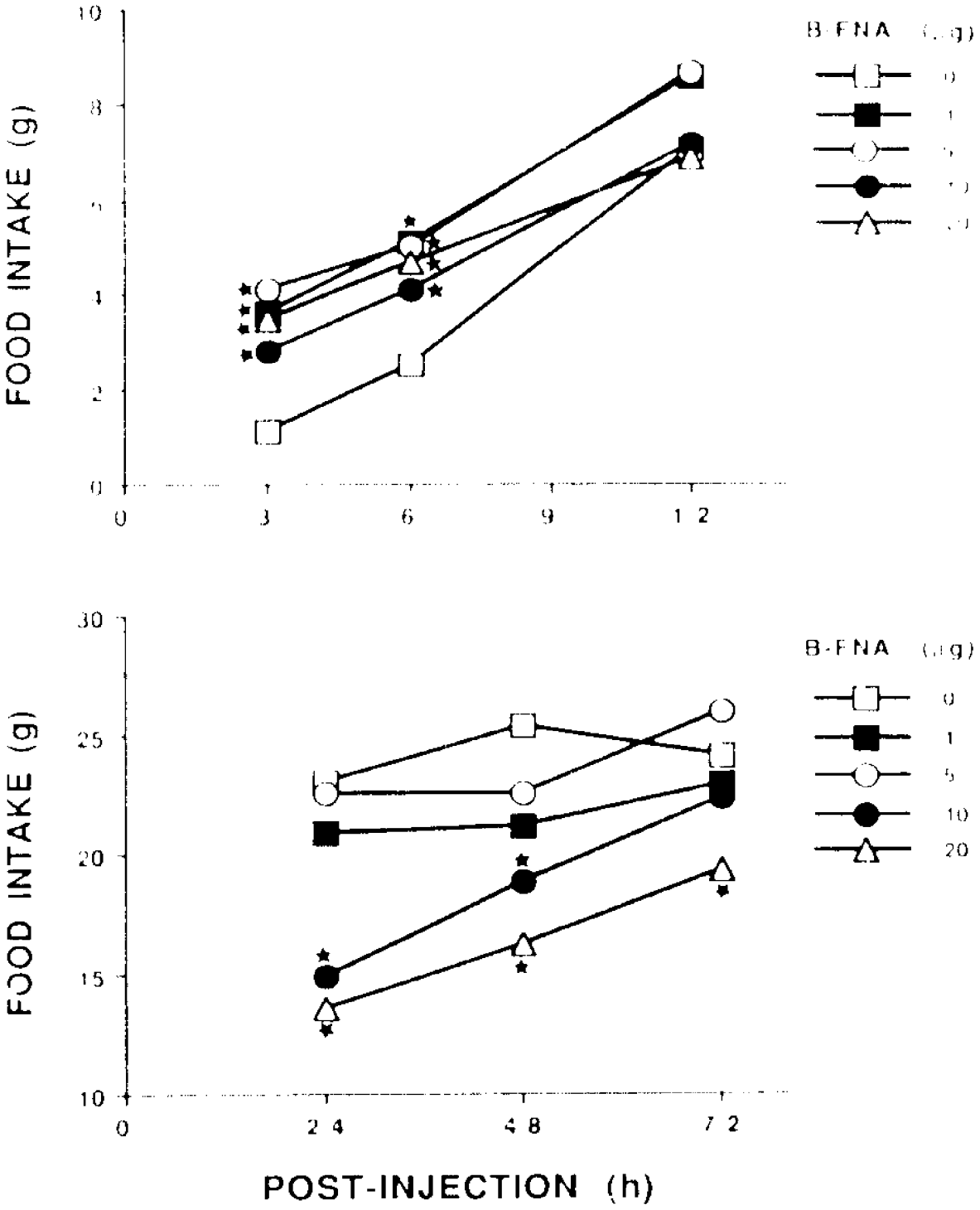

FOOD INTAKE (g)
10
8
4
2
0
0
3
6
9
12
B-FNA (μg)
0
1
5
10
20
FOOD INTAKE (g)
30
25
20
15
10
0
24
48
72
B-FNA (μg)
0
1
5
10
20
POST-INJECTION (h)

Table 1. Alterations in body weight (g, S.E.M.) following central administration of beta-funaltrexamine (β-FNA).

β-FNA dose (μg)	Post-injection (h) 24	48	72
0	- 2.1 (1.6)	+ 3.9 (1.9)	+ 6.3 (1.6)
1	- 3.2 (4.1)	+ 2.0 (2.7)	+ 4.8 (1.9)
5	- 4.4 (3.9)	+ 4.4 (4.2)	+10.6 (3.1)
10	-20.6 (3.7)*	-12.0 (3.5)*	-8.0 (2.6)
20	-29.6 (5.7)*	-23.1 (7.9)*	-22.2(9.4)*

* Significant difference in β-FNA-induced body weight change relative to vehicle injections (Dunnett comparisons, $P<0.05$).

intake by 24% after 24h while the 20μg ß-FNA dose significantly suppressed intake by 29% after 24h and by 20% after 48h. Similar to free feeding, the effects of ß-FNA upon deprivation-induced feeding were dose-dependent and time-dependent with the higher ß-FNA dose producing greater and more prolonged effects.

Significant differences were noted in body weight following vehicle and ß-FNA treatment. Body weight gain was significantly suppressed by ß-FNA in the food deprived rats, a response which paralleled that in freely-feeding rats (data not shown).

1C. Glucoprivic Feeding

Figure 3 displays significant differences in glucoprivic feeding among vehicle, 2DG and ß-FNA/2DG conditions at 2h ($F=9.24$, $P<0.0006$), 4h ($F=12.03$, $P<0.0001$) and 6h ($F=7.35$, $P<0.0023$), but not at 24h ($F=1.36$) following systemic injection of 2DG. 2DG significantly increased food intake following central pretreatment with vehicle by 300% at 2h, by 300% at 4h, and by 210% at 6h. Pretreatment with ß-FNA suppressed the compensatory increase in consumption induced by glucoprivation, with significant reductions demonstrated with both ß-FNA doses (indicated by the stars). The 10μg dose of ß-FNA inhibited intake by 75% at 2h, by 52% at 4h, and by 56% at 6h, while the 20μg dose of ß-FNA decreased 2DG feeding by 100% at 2h, by 87% at 4h and by 78% at 6h. Similar to the free-feeding and food deprivation paradigms, ß-FNA significantly reduced body weight gain in glucoprivic rats (data not shown).

1D. ß-FNA/Nor-BNI Short-Term Effects

Figure 2. Alterations in deprivation-induced intake (g) following 24h pretreatment with β-FNA. Standard errors of the mean (S.E.M.) ranges at 2h (10μg: 0.9; 20μg: 0.8), 4h (10μg: 1.0; 20μg: 0.8), 24h (10μg: 2.4; 20μg: 2.1) and 48h (10μg: 1.6; 20μg: 0.9).

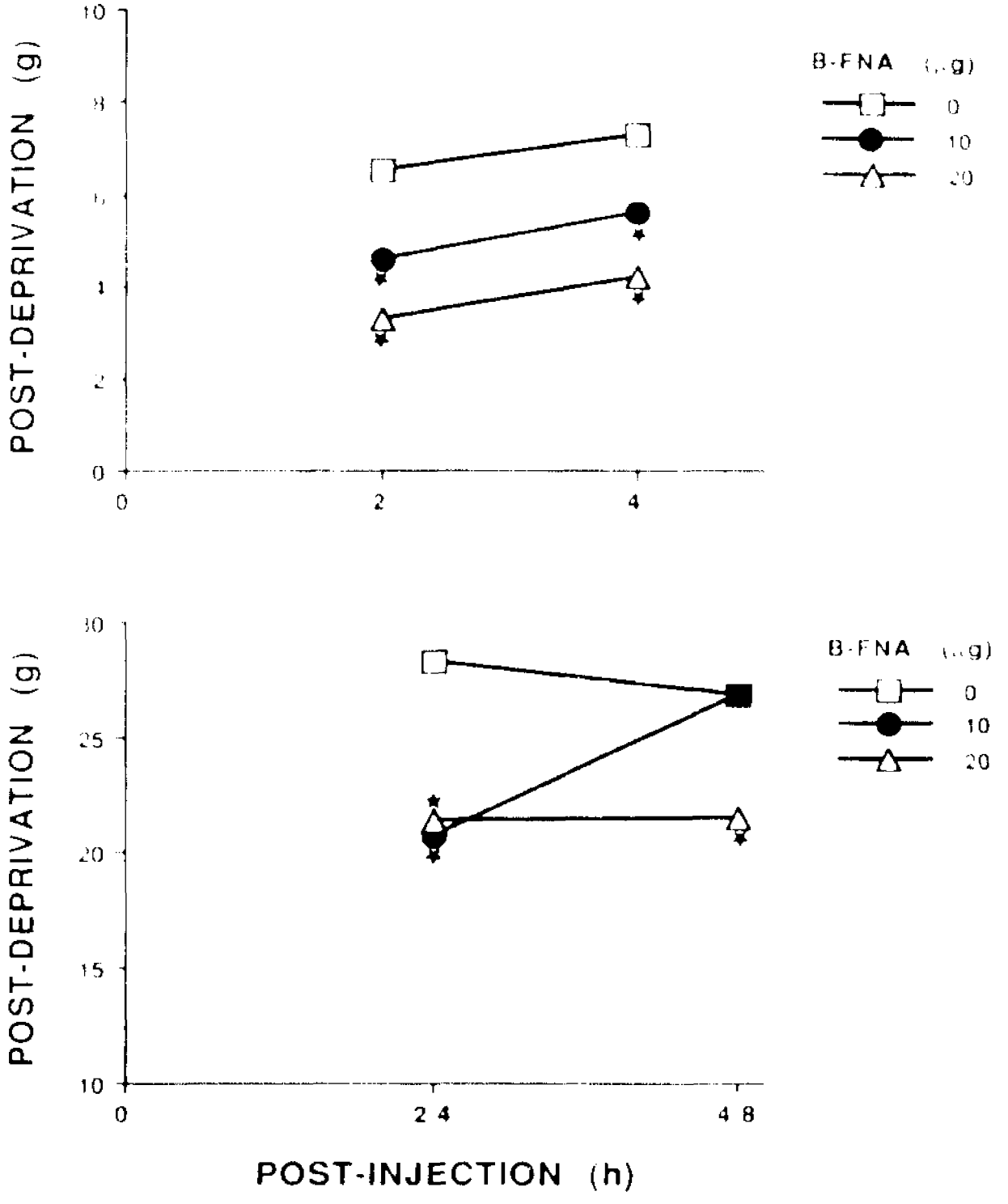

POST-DEPRIVATION (g)
10
8
6
4
2
0
0
2
4
B-FNA (µg)
0
10
20
POST-DEPRIVATION (g)
30
25
20
15
10
0
24
48
POST-INJECTION (h)
B-FNA (µg)
0
10
20

Figure 3. Alterations in 2-deoxy-D-glucose (2DG, 650 mg/kg, IP) hyperphagia in rats centrally pretreated (24h) with β-FNA. Standard errors of the mean (S.E.M.) ranges at 2h (0.2-0.5), 4h (0.3-0.7) and 6h (0.5-0.8).

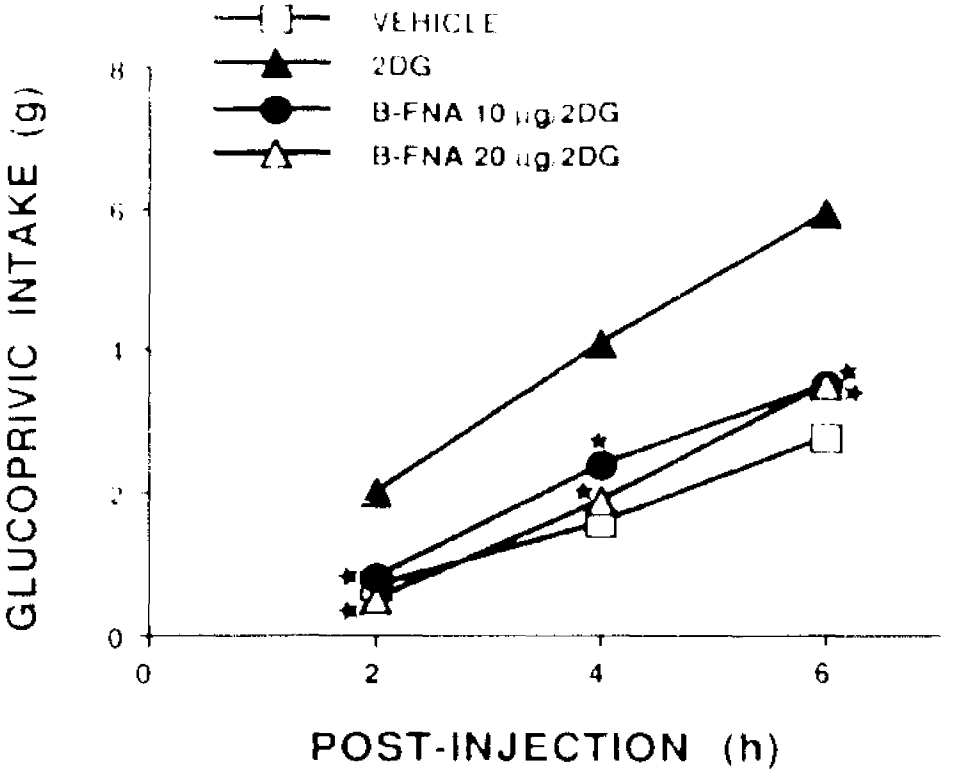
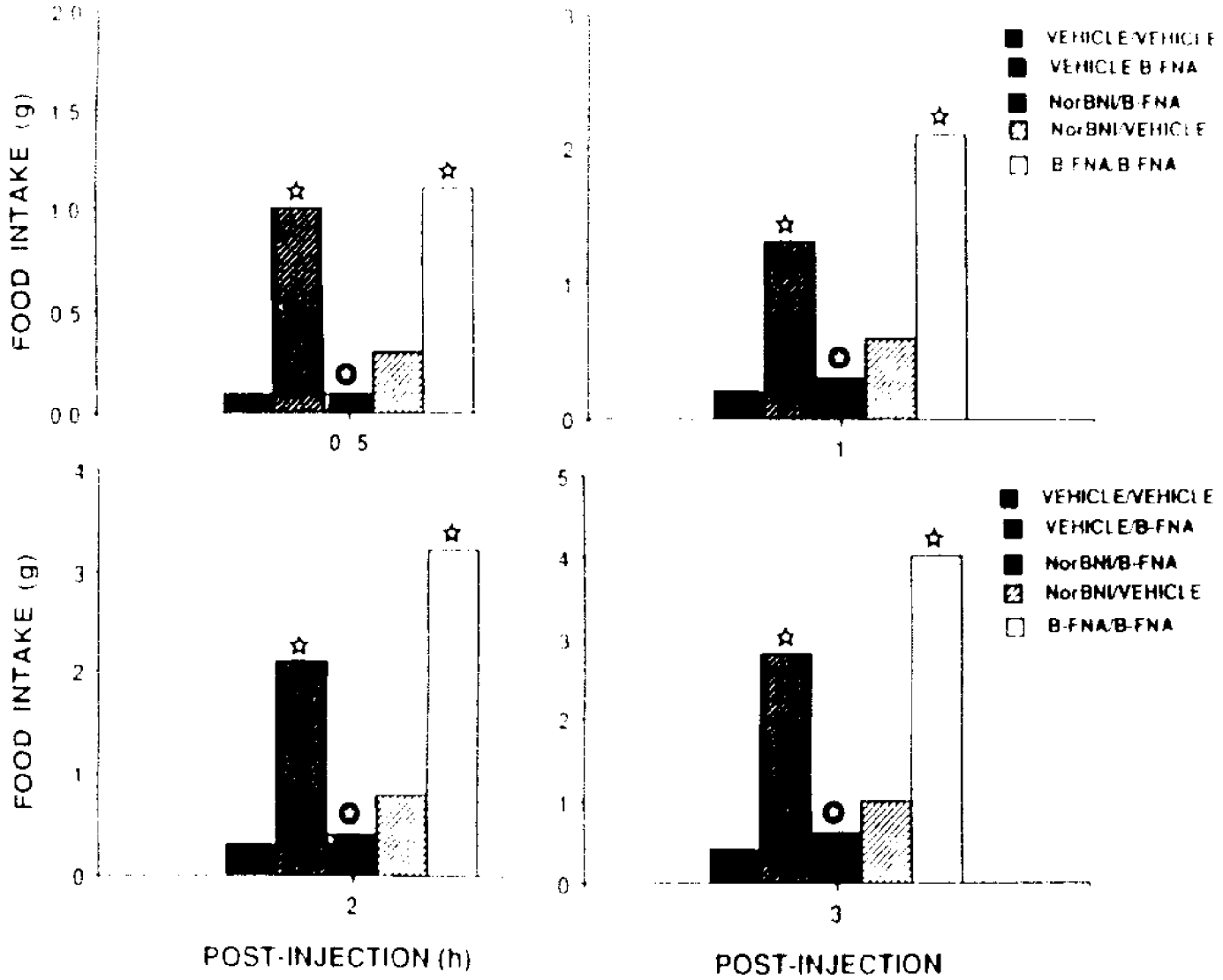

VEHICLE
2DG
B-FNA 10 µg 2DG
B-FNA 20 µg 2DG
GLUCOPRIVIC INTAKE (g)
0
2
4
6
POST-INJECTION (h)

Figure 4 indicates the significant differences of short-term β-FNA upon spontaneous intake among conditions at 0.5h ($F_{4,49}$=8.70, P<0.0001), 1h (F=12.66, P<0.0001), 2h (F=18.05, P<0.0001) and 3h (F=20.00, P<0.0001) after injection. As indicated by the opened stars, β-FNA significantly increased food intake across the 3-hour time course. Nor-BNI, administered 1h prior to β-FNA treatment, completely abolished the short-term stimulatory effect of β-FNA upon free feeding across the 3-h time course (denoted by the closed stars). Since Nor-BNI treatment failed to alter food intake relative to vehicle treatment, this response could not be attributed merely to the hypophagic effect of Nor-BNI itself. In contrast, β-FNA administered 24h prior to a second β-FNA injection, failed to alter the latter's short-term stimulatory effect upon intake across the 3h time course.

Figure 4. Alterations in short-term β-FNA-induced hyperphagia by pretreatment with either Nor-BNI or β-FNA across a 3h time course. Significant differences in food intake are denoted by open and close stars corresponding to vehicle/vehicle and vehicle/β-FNA conditions respectively (Dunnett comparisons, $P<0.05$). Standard errors of the mean (S.E.M.) ranges at 0.5h (0.03-0.26), 1h (0.07-0.39), 2h (0.07-0.59) and 3h (0.08-0.57).


FOOD INTAKE (g)
0 5
1
2
3
POST-INJECTION (h)
POST-INJECTION
VEHICLE/VEHICLE
VEHICLE/B-FNA
NorBNI/B-FNA
NorBNI/VEHICLE
B-FNA/B-FNA

Experiment 2: Nor-binaltorphimine and Ingestive Behavior

2A. Nocturnal Intake Protocol

A maximum of four microinjection conditions were administered at weekly intervals: (a) vehicle (10μl) (n=10), Nor-BNI at doses of (b) 1μg (n=8), (c) 10μg (n=10), (d) 20μg (n=10) and (e) naltrexone (20μg) (n=7). Food intake was assessed at 1, 2 and 12h into the dark cycle. Naltrexone was used because it binds non-selectively to opiate receptors for a longer duration than naloxone (Gray and Robinson, 1974). Drugs were administered 1h prior to the onset of the dark cycle to allow binding to the appropriate opiate binding site.

2B. High Fat Diet Protocol

Four to five hours into the light cycle, seven rats received a total of five microinjections at weekly intervals which were administered 1 hour prior to the introduction of a high fat diet: (a) vehicle, Nor-BNI at doses of (b) 1μg, (c) 10μg, (d) 20μg and (e) naltrexone (20μg). Palatable food intake was assessed at 1 and 2h following each injection, adjusting for spillage by placement of paper under each wire cage and collecting and measuring the food particles. Fresh servings of the diet was administered at 120h intervals, alternating between injections and non-injections to determine whether the animals returned to baseline levels of intake.

2C. Glucoprivic Feeding Protocol

Cannulated rats received a total of five injection conditions at weekly intervals at 1 to 2 hours into the light cycle: (a) vehicle (10μl, ICV)-vehicle (1.5ml normal saline/kg body weight, IP, n=10), (b) vehicle-2DG (650mg/kg, IP, n=10), (c) Nor-BNI (20μg, ICV)-vehicle (n=8), (d) Nor-BNI (20μg, ICV)-2DG

(n=10) and (e) naltrexone (20μg, ICV)-2DG (n=10). A period of 1 hour was allowed to elapse between central and peripheral injections. Food intake was determined 2, 4, 6 and 24 hours following the systemic injection. Body weight was measured immediately prior to and 24 hours after each injection.

Results

2A. Nocturnal Intake

Figure 5 illustrates the significant differences in nocturnal feeding among vehicle, Nor-BNI and naltrexone (NTX) conditions at 1h ($F_{4,40}=3.30$, $P<0.02$) and 2h ($F=3.51$, $P<0.015$) after the onset of the dark cycle. Nor-BNI (20μg) and NTX (20μg) significantly suppressed nocturnal feeding by 53-54% and 47-60%, respectively (denoted by the stars). Although the lower doses of Nor-BNI (1μg and 10μg) reduced nocturnal intake by 21-45%, these responses failed to reach significance. Nocturnal intake was significantly attenuated after 12h ($F=3.85$, $P<0.01$) by 43% following the 20μg dose of Nor-BNI, but not by the 20μg dose of NTX (data not shown).

2B. High-Fat Diet Intake

Indicated on Figure 6 are significant differences in high-fat diet intake among vehicle, Nor-BNI and NTX treatments at 1h ($F_{4,30}=7.94$, $P<0.0002$) and 2h ($F=11.89$, $P<0.0001$) after diet introduction. As denoted by the stars, Nor-BNI suppressed intake of the high-fat diet: 1μg (33% at both 1 and 2h), 10μg (43% at 1h and 41% at 2h) and 20μg (80% at 1h and 79% at 2h). NTX (20μg) also significantly reduced high-fat diet intake by 50% at 1h and by 47% at 2h.

Figure 5. Alterations of nocturnal food intake during the first two hours of the dark phase in freely-feeding rats centrally pretreated with Nor-BNI or naltrexone (NTX). The closed stars denote significant decreases in nocturnal intake (Dunnett comparisons, $P<0.05$) relative to corresponding vehicle values.

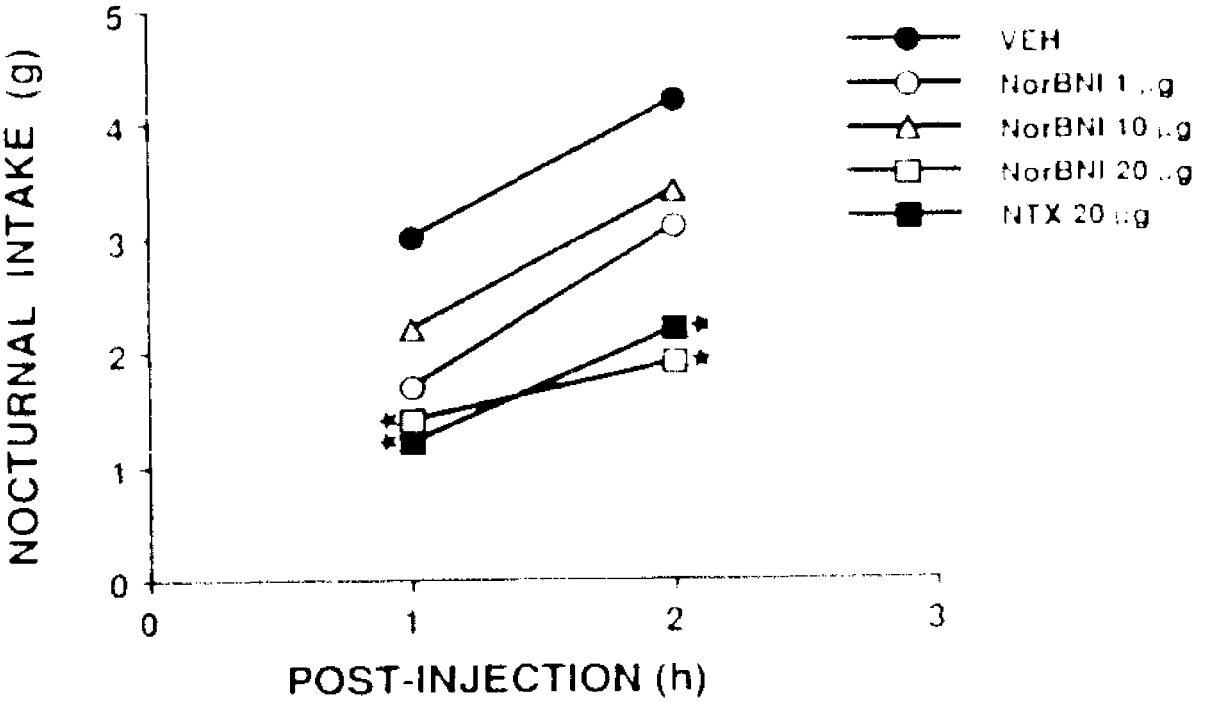
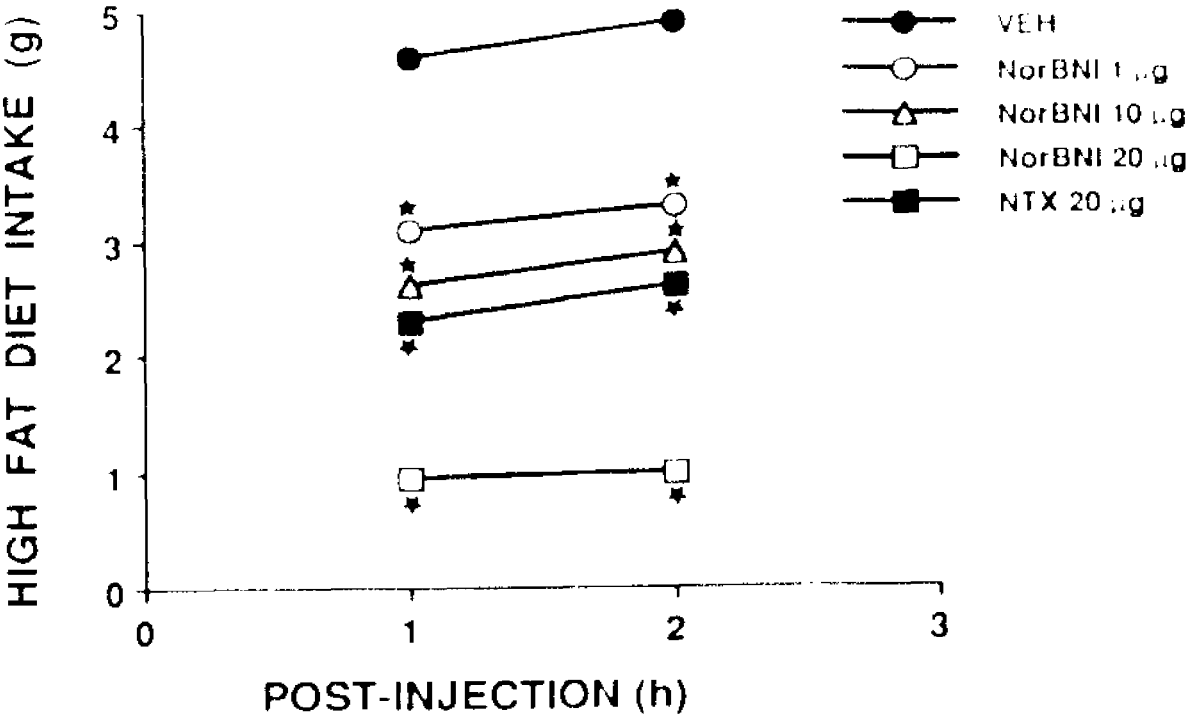

NOCTURNAL INTAKE (g)
POST-INJECTION (h)
0
1
2
3
4
5
0
1
2
3
VEH
NorBNI 1 µg
NorBNI 10 µg
NorBNI 20 µg
NTX 20 µg

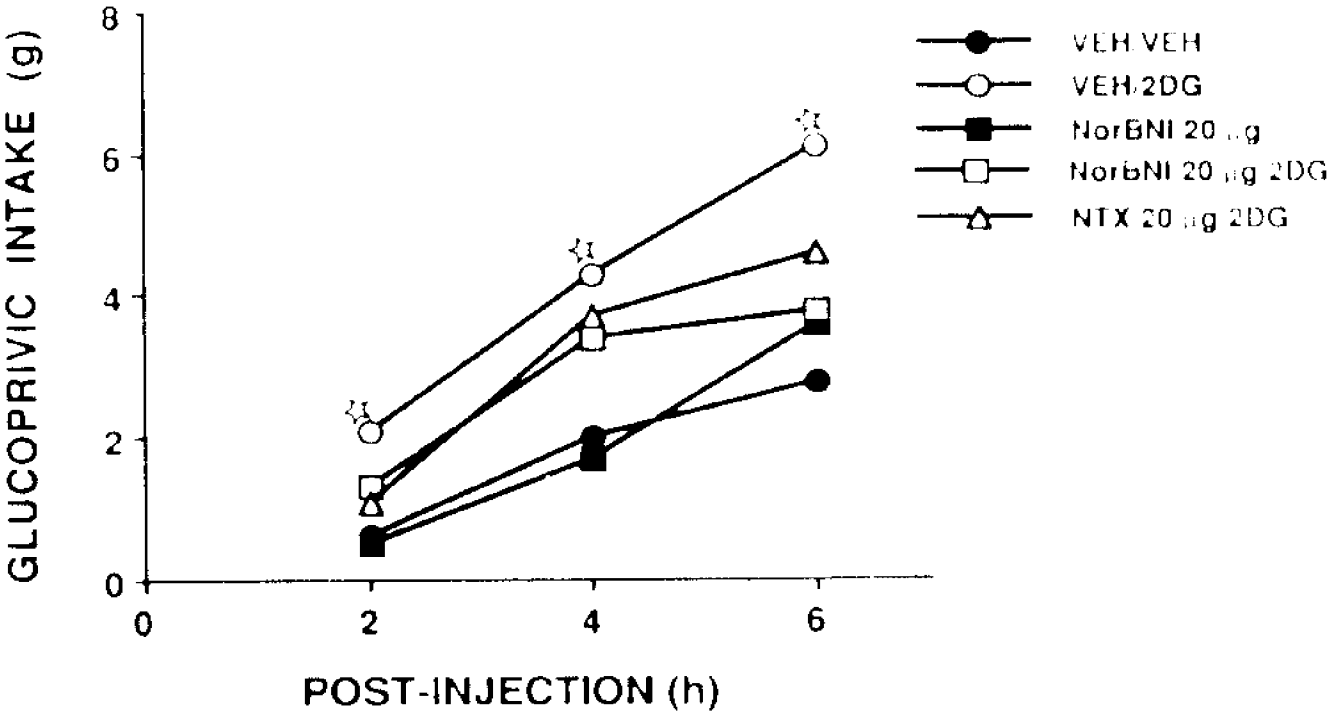
Figure 6. Alterations in intake of a high-fat diet following central pretreatment with Nor-BNI or NTX. The closed stars indicate significant suppression in high-fat diet intake (Dunnett comparisons, $P<0.05$) relative to corresponding vehicle values.


HIGH FAT DIET INTAKE (g)
5
4
3
2
1
0
0
1
2
3
POST-INJECTION (h)
VEH
NorBNI 1 µg
NorBNI 10 µg
NorBNI 20 µg
NTX 20 µg

2C. Glucoprivic Feeding

Figure 7 notes the significant differences in glucoprivic intake among conditions at 2h ($F_{4,43}$=5.32, P<0.01), 4h (F=7.31, P<0.0001) and 6h (F=3.79, P<0.01), but not at 24h (F=1.10) following injection. Indicated by the stars, 2DG significantly increased food intake across the 6-hour time course. NTX pretreatment significantly prevented the occurrence of 2DG hyperphagia, and reduced 2DG intake by 28-69%. Nor-BNI pretreatment also prevented the occurrence of 2DG hyperphagia and reduced 2DG intake by 40-68% over the 6h time course. However, Nor-BNI itself failed to alter basal food intake relative to vehicle injections.

Figure 7. Alterations in 2DG hyperphagia in rats centrally pretreated (1h) with Nor-BNI or NTX. The open stars indicate significant increases in food intake (Dunnett comparisons, $P<0.05$) relative to corresponding vehicle values.


GLUCOPRIVIC INTAKE (g)
8
6
4
2
0
0
2
4
6
POST-INJECTION (h)
VEH:VEH
VEH:2DG
NorBNI 20 µg
NorBNI 20 µg 2DG
NTX 20 µg 2DG

Experiment 3: DALCE and Ingestive Behavior

3A. Free Feeding Protocol

A maximum of four microinjection conditions were administered at the beginning of the light cycle at weekly intervals: (a) vehicle (10μl, n=22), DALCE at doses of (b) 1μg (n=7), (c) 10μg (n=7), (d) 20μg (n=16) and (e) 40μg (n=11). Food intake was measured 2, 4, 6, 10, 24, 48, 72 and 96 hours after each injection. Body weight was determined prior to, and at 24, 48, 72 and 96 hours following each injection.

3B. Food Deprivation Protocol

A total of five microinjection conditions were administered 1 to 2 hours into the light cycle at weekly intervals: (a) vehicle (10μl, n=18), DALCE at doses of (b) 1μg (n=8), (c) 10μg (n=8), (d) 20μg (n=18) and (e) 40μg (n=9). Rats received their particular injection and were then deprived of food, but allowed access to tap water ad libitum for 24h. Food was reintroduced and intake was assessed 2, 4, 24, 48 and 72 hours thereafter. Body weight was determined immediately prior to, and at 24, 48 and 72 hours after each injection.

3C. Glucoprivic Feeding Protocol

The following microinjection conditions were administered 1 to 2 hours into the light cycle at weekly intervals: (a) vehicle (10μl, ICV)-vehicle (1.5 ml saline/kg body weight, IP, n=15), (b) vehicle (10μl, ICV)-2DG (650 mg/kg, IP, n=15), (c) DALCE (1μg, ICV)-2DG (n=11), (d) DALCE (10μg, ICV)-2DG (n=12) and (e) DALCE (20μg, ICV)-2DG (n=15). A period of twenty-four hours was allowed to elapse between the central and peripheral injections in order for antagonist effects to occur at the delta binding sites. The measurement of food intake

occurred at 2, 4, 6 and 24 hours after peripheral injections. Body weight was measured immediately prior to and at 24 and 48 hours after injection.

3D. High Fat Diet Protocol

As in Experiment 2B, microinjections were administered 24h prior to the introduction of the high-fat diet 4 to 6h into the light cycle: (a) vehicle (10μl, n=12), DALCE at doses of (b) 1μg (n=7), (c) 10μg (n=11) and (d) 20μg (n=11). Food intake was assessed 1 and 2h following introduction of the diet and adjusted for spillage.

3E. Opioid Antagonists and DALCE Hyperphagia Protocol

The following microinjection conditions were administered 1 to 2 hours into the light cycle at weekly intervals: (a) vehicle (10μl, ICV)-vehicle (10μl, ICV) (n=26), (b) vehicle-DALCE (10μg, ICV) (n=26), (c) Nor-BNI (20μg, ICV)-DALCE (10μg, ICV) (n=12), (d) naltrexone (20μg, ICV)-DALCE (10μg, ICV) (n=13), (e) β-FNA (20μg, ICV)-DALCE (10μg, ICV) (n=12) and (f) DALCE (40μg, ICV)-DALCE (10μg, ICV) (n=13). A period of 1h was allowed to elapse between the first and second injections of the first four conditions to allow reversible antagonist binding. β-FNA and DALCE were administered 24 hours before the second DALCE injection to allow irreversible binding. Food intake, accounting for spillage, was determined 2, 4, 6,10, and 24 hours following the second injection.

Results

3A. Free Feeding

Figure 8 demonstrates the effect of different doses of DALCE upon free feeding for the first 10 hours (upper panel) and for 24 72 hours (lower panel)

following microinjection. The stars on the upper portion of the figure indicate significant increases in free feeding following the 10μg dose of DALCE by 77% at 4h ($F_{4,58}$=3.53, P<0.012), by 69% at 6h (F=4.52, P<0.003) and by 46% at 10h (F=1.93) after central injection. As noted on the lower panel, DALCE failed to alter spontaneous intake at 24h ($F_{4,58}$=1.06), 48h (F=0.46), 72h (F=1.61) and 96h (F=1.18) (data not shown) after microinjection. However, DALCE transiently, but nonsignificantly reduced free feeding by 20% 24h following the 40μg dose, but failed to alter subsequent intake.

Table 2 summarizes the significant changes in body weight during free feeding among vehicle and DALCE conditions ($F_{4,58}$=7.64, P<0.0001) and across the time course ($F_{4,232}$=23.27, P<0.0001). Vehicle-treated rats failed to display changes in body weight at 24h and 48h after injection, but significantly increased body weight at 72h and 96h following injection. However, DALCE produced a significant and dose-dependent reduction in body weight at 24h after injection and reduced the subsequent body weight gain observed in vehicle-treated animals. A ratio of body weight/food intake was determined across dose and time condition to evaluate whether DALCE was altering feeding efficiency. Significant differences in feeding efficiency were demonstrated across the time course ($F_{3,174}$=5.63, P<0.001), and for the interaction between conditions and times ($F_{12,174}$=2.21, P<0.013), but not among conditions ($F_{4,58}$=1.65). Significant changes in feeding efficiency only occurred at 24h following the highest dose of DALCE.

3B. Deprivation-induced Feeding

Noted on Figure 9 are the effects of different doses of DALCE upon deprivation-induced feeding for the first 4 hours (upper panel) and for 24-72h (lower panel) following the reintroduction of food. DALCE failed to produce

Figure 8. Alterations in food intake (g) following central administration of DALCE. The stars indicate significant differences in food intake following given doses of DALCE relative to vehicle at each corresponding time point. Standard errors of the mean (S.E.M.) across conditions at 2h (0.2-0.7), 4h (0.3-0.6), 6h (0.3-0.9), 10h (0.5-1.6), 24h (0.5-3.3), 48h (0.6-2.2) and 72h (0.7-1.6).

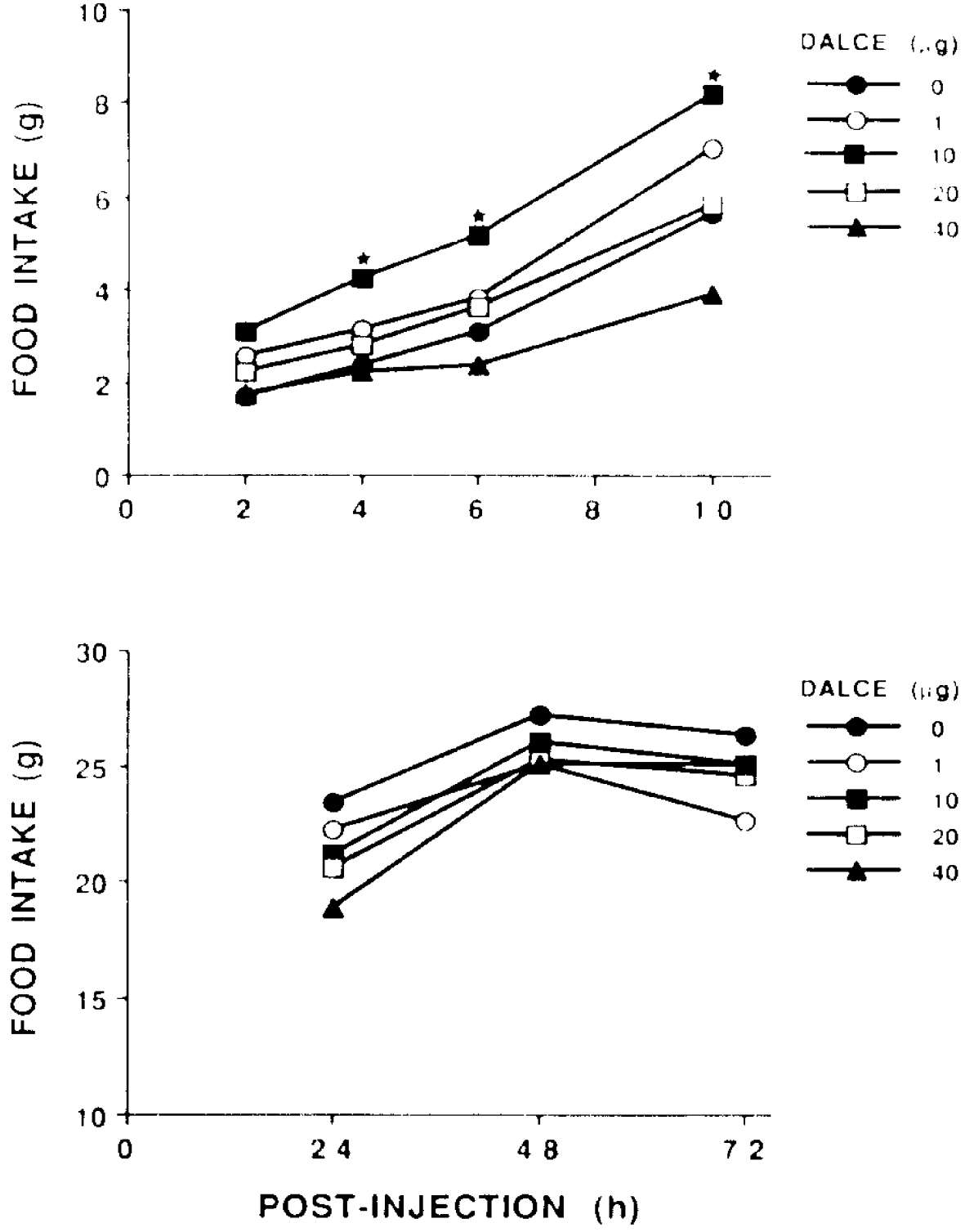

FOOD INTAKE (g)
10
8
6
4
2
0
0
2
4
6
8
10
DALCE (μg)
0
1
10
20
40
FOOD INTAKE (g)
30
25
20
15
10
0
24
48
72
POST-INJECTION (h)
DALCE (μg)
0
1
10
20
40

Table 2. Time-related alterations in body weight change (g, S.E.M.) following vehicle and [D-Ala^2,Leu^5,Cys^6]-Enkephalin (DALCE) relative to pre-injection values in freely feeding rats.

DALCE dose (μg)	Post-injection (h) 24	48	72	96
0	-4.1(0.5)	+4.6(0.1)	+8.8(1.1)*	+11.7(1.0)*
1	-9.9(1.4)*	-3.3(3.1)	+0.6(2.7)	-2.6(1.9)
10	-8.2(0.1)*	-1.2(0.3)	+1.8(0.7)	+2.0(0.6)
20	-12.9(1.0)*	-2.2(0.4)	0.0(0.9)	+0.9(1.6)
40	-18.1(0.8)*	-6.5(0.9)	-3.9(0.3)	+0.5(1.1)

* Significant changes in body weight relative to corresponding pre-injection values (Dunnett comparisons, $P<0.05$).

significant differences in deprivation-induced feeding at 2h ($F_{4,56}$=0.61), 4h (F=0.31) and 24h (F=0.52) after food was reintroduced. However, DALCE significantly reduced food intake at 48h (F=2.75, P<0.037) and 72h (F=5.07, P<0.002) following the reintroduction of food (denoted by the stars). The significant long-term decreases of intake following DALCE administration were not dose-dependent in that inhibition occurred only following the 1μg dose at 48h (15% reduction) and the 10μg dose at 48h (13% reduction) and 72h (15% reduction).

Table 3 summarizes the significant changes in body weight during deprivation-induced feeding across the time course ($F_{4,224}$=284.94, P<0.0001) and for the interaction between conditions and times ($F_{16,224}$=2.03, P<0.013), but not among conditions ($F_{4,56}$=2.13). DALCE failed to alter weight loss incurred after 24h of food deprivation. Whereas the 40μg dose of DALCE significantly retarded body weight recovery 24h after food reintroduction, body weight recovery was significantly facilitated after food reintroduction following the 10μg (48h) and 20μg (48 and 72h) doses of DALCE.

3C. Glucoprivic Feeding

Figure 10 illustrates the significant differences in glucoprivic feeding among vehicle, 2DG and DALCE/2DG conditions at 2h ($F_{4,65}$=4.61, P<0.0025), 4h (F=14.07, P<0.0001) and 6h (F=14.23, P<0.0001), but not at 24h (F=0.83) following systemic injection of 2DG. 2DG significantly increased food intake by 244% at 2h, by 343% at 4h and by 241% at 6h. Whereas DALCE (10μg) pretreatment significantly suppressed 2DG hyperphagia by 30% at 2h (denoted by the stars), DALCE (1μg) pretreatment potentiated 2DG hyperphagia by 55% at 4h and by 35% at 6h.

Figure 9. Alterations in deprivation-induced intake (g) in rats treated 24h earlier with DALCE. The stars indicate significant differences in food intake following given doses of DALCE relative to vehicle at each corresponding time point (Dunnett comparisons, $P<0.05$). Standard errors of the mean (S.E.M.) ranges at 2h (0.5-1.3), 4h (0.5-1.4), 24h (1.2-4.8), 48h (0.8-1.9) and 72h (0.5-1.7).

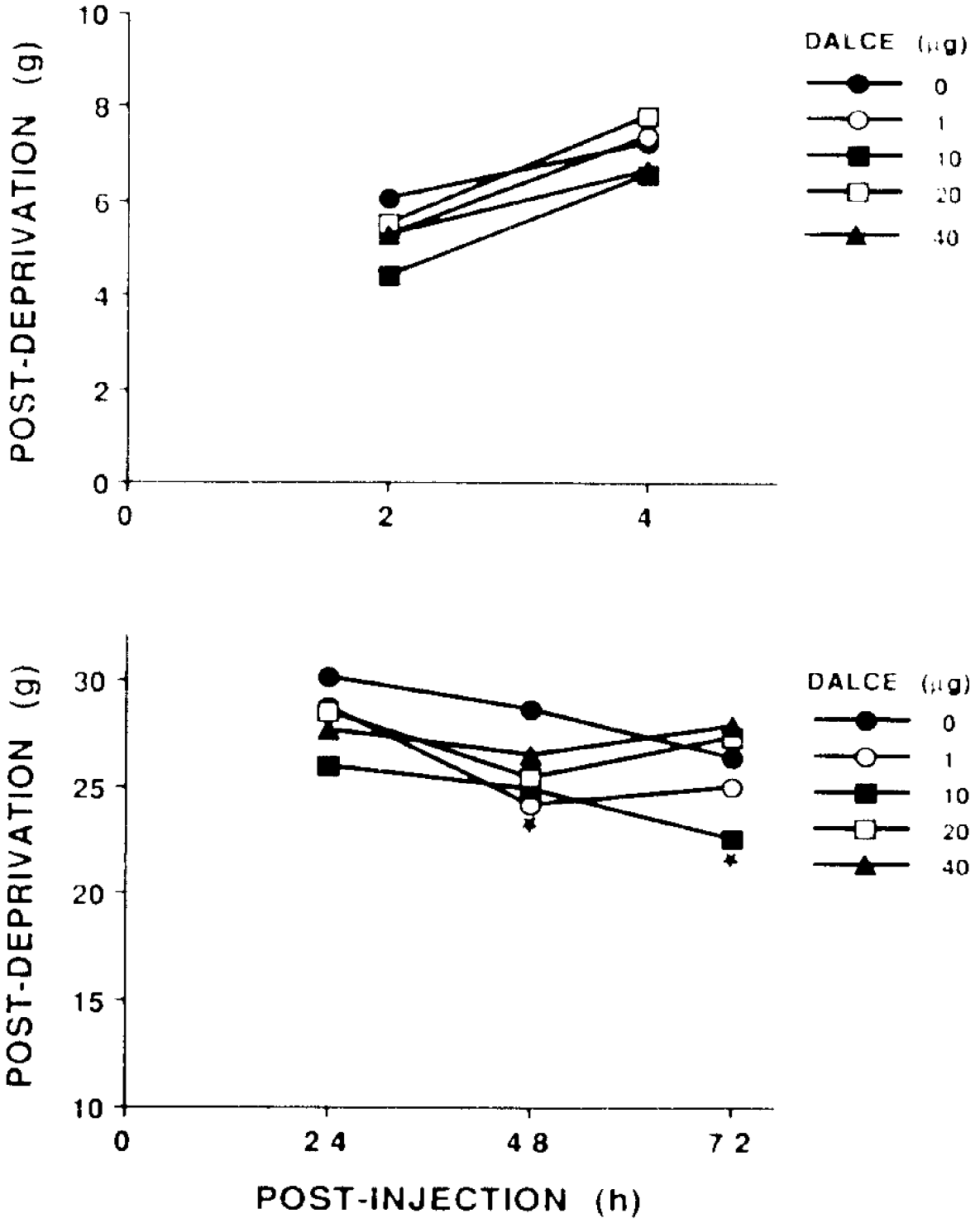
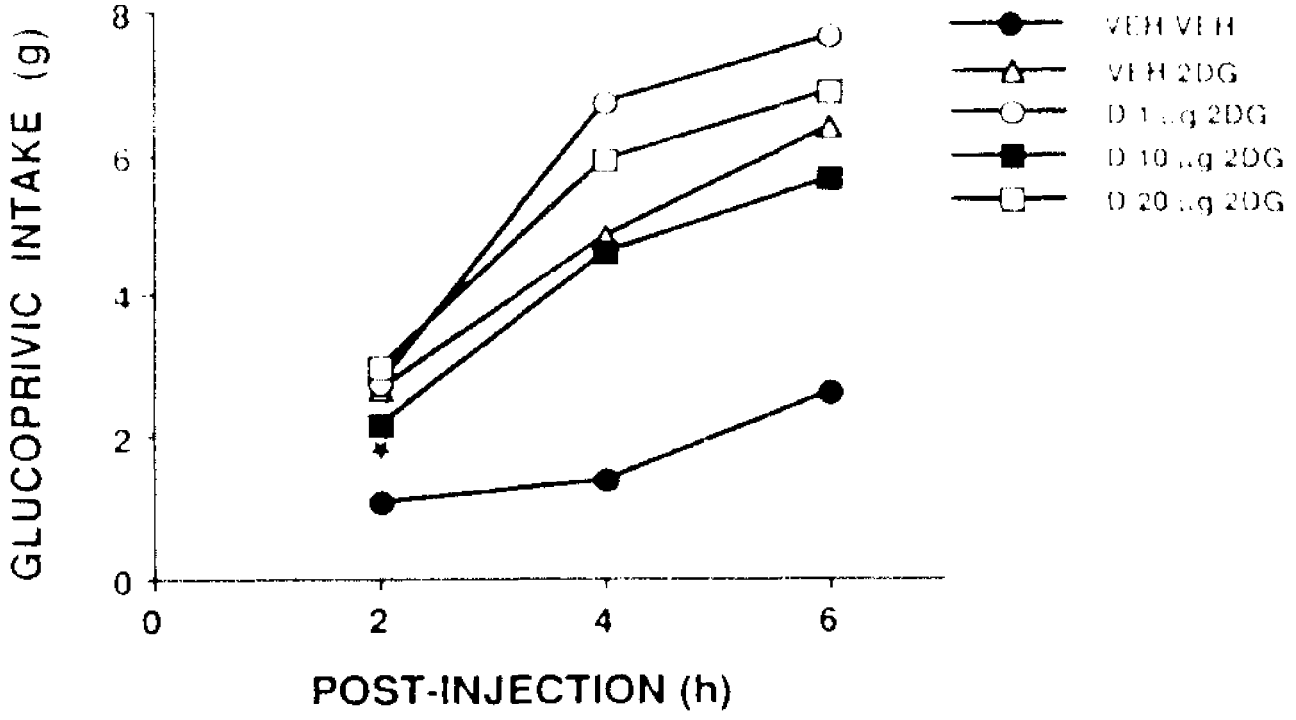

POST-DEPRIVATION (g)
10
8
6
4
2
0
0
2
4
DALCE (µg)
0
1
10
20
40
POST-DEPRIVATION (g)
30
25
20
15
10
0
24
48
72
POST-INJECTION (h)
DALCE (µg)
0
1
10
20
40

Table 3. Time-related alterations in body weight change (g, S.E.M.) following vehicle and [D-Ala^2,Leu^5,Cys^6]-Enkephalin (DALCE) relative to pre-deprivation values in food-deprived rats.

DALCE dose (μg)	Post-deprivation	Post-reintroduction (h) 24	48	72
0	-40.8(5.4)	-9.0(5.6)	-7.4(5.5)	-3.7(5.8)
1	-34.8(7.8)	-8.0(8.3	-4.0(8.0)	-1.5(8.0)
10	-37.0(5.9)	-4.7(6.6)	-0.3(7.3)*	+1.4(6.5)
20	-38.9(6.9)	-6.3(6.4)	+1.3(6.1)*	+5.5(6.1)*
40	-43.0(11.3)	-20.0(16.4)*	-9.4(13.4)	-2.0(12.3)

* Significant changes in body weight relative to corresponding vehicle injection values (Dunnett comparisons, $P<0.05$).

Figure 10. Alterations in 2DG hyperphagia in rats pretreated 24h earlier with DALCE. The stars indicate significant differences in food intake following given doses of DALCE relative to vehicle at each corresponding time point (Dunnett comparisons, $P<0.05$).


GLUCOPRIVIC INTAKE (g)
8
6
4
2
0
0
2
4
6
POST-INJECTION (h)
VEH VEH
VEH 2DG
D 1 µg 2DG
D 10 µg 2DG
D 20 µg 2DG

3D. High-Fat Diet Intake

Noted on Figure 11 are the effects of different doses of DALCE upon high-fat diet intake. Long-term pretreatment (24h) with DALCE 1μg dose significantly increased high-fat intake at 1h ($F_{3,37}$=5.85, P<0.002) and 2h (F=3.67, P<0.023) after exposure to the diet (indicated by the stars).

3E. DALCE and Short-term Antagonist Effects

Figure 12 illustrates the significant differences in free feeding among conditions at 2h ($F_{5,96}$=15.83, P<0.0001), 4h (F=14.12, P<0.0001), 6h (F=14.49, P<0.0001) and 10h (F=11.69, P<0.0001) following the second vehicle or DALCE injection. DALCE 10μg dose significantly increased food intake following central pretreatment with vehicle across the 10-hour time course. Pretreatment (1h) with the general opiate antagonist, NTX, significantly inhibited DALCE hyperphagia by 87% at 2h, by 84% at 4h, by 100% at 6h and by 100% at 10h. Pretreatment (1h) with the kappa antagonist, Nor-BNI, significantly inhibited DALCE hyperphagia by 100% at 2h, by 85% at 4h, by 100% at 6h and by 100% at 10h. In contrast, the mu antagonist, β-FNA, administered 24h prior to DALCE treatment, transiently and nonsignificantly altered DALCE hyperphagia by decreasing intake by 39% at 2h and by 26% at 10h. DALCE (40μg) pretreatment (24h) failed to significantly alter subsequent DALCE hyperphagia across the 10-hour time course.

Figure 11. Alterations in intake (g) of a high-fat diet over 2h in rats pretreated with DALCE. The stars indicate significant differences in food intake following given doses of DALCE relative to vehicle at each corresponding time point (Dunnett comparisons, $P<0.05$). Standard errors of the mean (S.E.M.) ranges at 1h (0.3-0.7) and 2h (0.4-0.6).

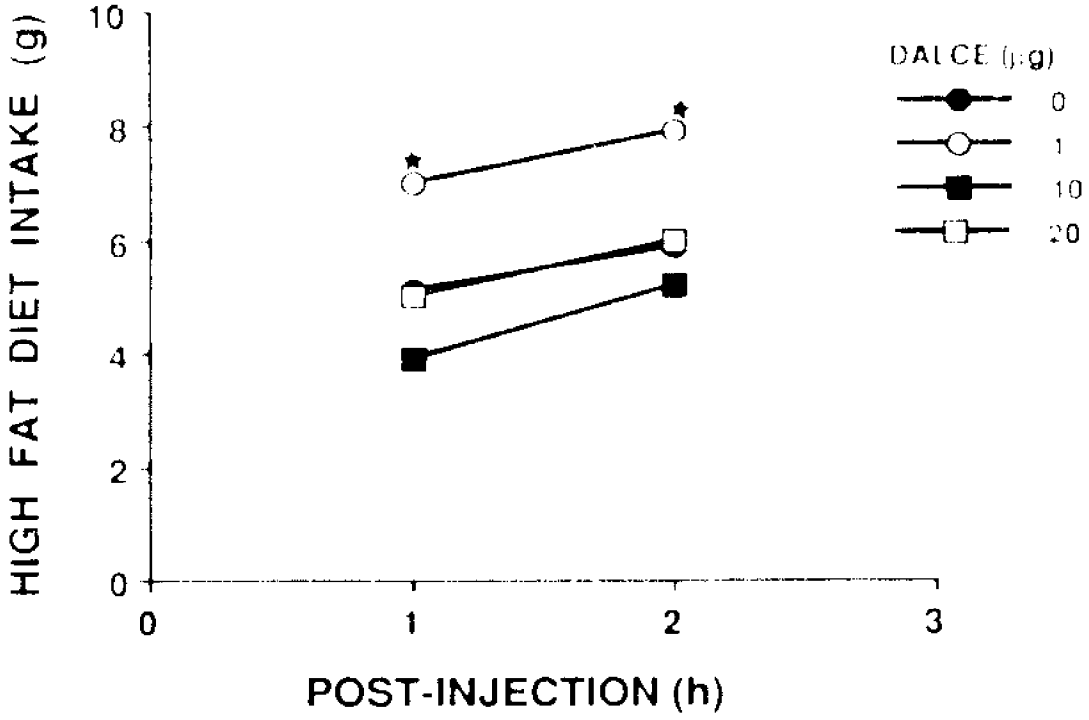
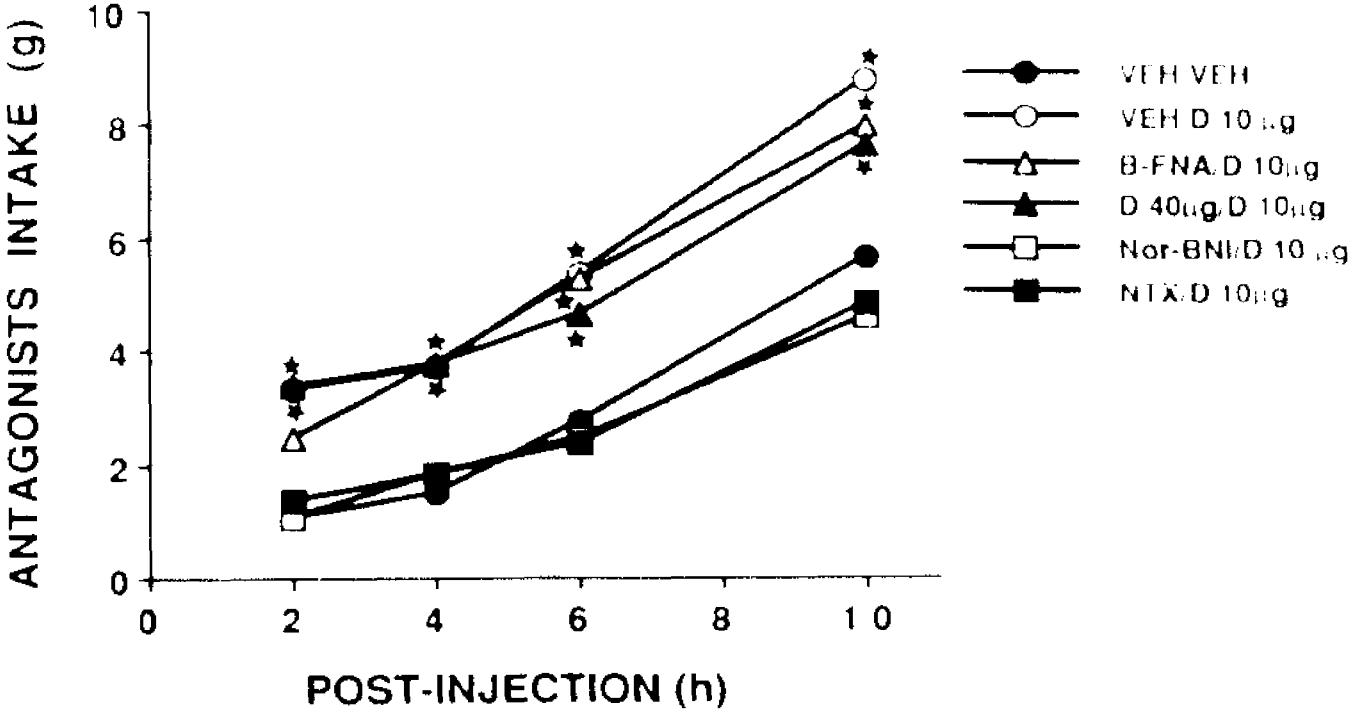

HIGH FAT DIET INTAKE (g)
10
8
6
4
2
0
0
1
2
3
POST-INJECTION (h)
DALCE (μg)
0
1
10
20

Figure 12. Alterations in DALCE (10μg) hyperphagia in rats pretreated with Nor-BNI, NTX, β-FNA or DALCE. The stars denote significant differences in food intake following given doses of DALCE relative to vehicle at each corresponding time point (Dunnett comparison, $P<0.05$). Standard errors of the mean (S.E.M.) ranges at 2h (0.2-0.4), 4h (0.1-0.4), 6h (0.3-0.6) and 10h (0.3-0.8).


ANTAGONISTS INTAKE (g)
10
8
6
4
2
0
0
2
4
6
8
10
POST-INJECTION (h)
VEH VEH
VEH D 10 µg
B-FNA D 10µg
D 40µg/D 10µg
Nor-BNI/D 10 µg
NTX/D 10µg

Discussion

The endogenous opioid system has long been implicated in the regulation of food intake, with agonists typically stimulating feeding and antagonists inhibiting food intake (Morley et al., 1983a). The general opiate receptor antagonists, naloxone and naltrexone, inhibit food intake under a variety of conditions, including spontaneous feeding (Carey et al., 1981; Cooper, 1980; Lowy et al., 1980; Sanger & McCarthy, 1981), deprivation intake (Brands et al., 1979; Brown & Holtzman, 1979; Holtzman, 1974), 2DG glucoprivation (Lowy et al., 1980; Sewell & Jawaharlal, 1980), intake of palatable diets (Apfelbaum & Mandenoff, 1981; Cooper et al., 1985) and nocturnal feeding (Brands et al., 1979; Cooper, 1980; Lowy et al., 1980). Similarly, long-acting general opiate antagonists, such as β-CNA and the trans-3,4-dimethyl-4-phenylpiperidine significantly reduce in food intake and body weight (Leander et al., 1982; Ward et al., 1982a). Since these antagonists lacked the ability to differentiate among the specific opiate receptor subtypes, the present study evaluated the ingestive effects of the specific mu antagonist, β-FNA, the specific kappa antagonist, Nor-BNI and the specific delta antagonist, DALCE. The following sections will discuss the findings of the three series of experiments which employed the specific antagonists, β-FNA, Nor-BNI and DALCE, under: A) free feeding, B) nocturnal intake, C) deprivation-induced feeding, D) glucoprivation and E) palatable intake.

A. Free Feeding

The effects of β-FNA upon spontaneous intake were largely consistent with previous observations (Ukai & Holtzman, 1988). The present study demonstrated that intracerebroventricular administration of β-FNA stimulated

short-term feeding for up to 6h with all ß-FNA doses (1-20μg) producing significant effects. With respect to time course and effective dose, the present data differ somewhat from the previous report (Ukai & Holtzman, 1988) which demonstrated significant increases in feeding at 0.5-2h following central administration following the 5μg, but not the 1.0 or 2.5μg, doses of ß-FNA. The increased feeding observed after 2h following all doses of ß-FNA in their study might have proven to be significant if longer ingestive intervals were employed.

The present study also found that ß-FNA (10-20μg) significantly inhibited free feeding by 35-41% after 24-72h, but not following the lower (1 and 5μg) doses. Both the present and previous studies found that body weight loss occurred in conjunction with the reduction of food intake. Given the similar temporal pattern of ß-FNA's inhibition of free feeding and its irreversible inhibition of mu receptors (Takemori et al., 1981), it appears that the long-term inhibition in feeding is due to ß-FNA's antagonism at mu receptors. The gradual recovery of food intake and body weight over the 72h time course following central injection appears to reflect a similar recovery of non-occupied mu receptors. The degree of inhibition observed with ß-FNA was similar to that demonstrated by the general opiate receptor antagonists, naloxone and naltrexone, despite their short-acting action (Cooper, 1980; Jalowiec et al., 1981). ß-CNA, while alkylating all opioid receptors, was also shown to reduce daily food intake and body weight gain, but on a long-term basis (2-4 days) (Gosnell et al., 1987). Furthermore, the trans-3,4-dimethyl-4-phenylpiperidine compounds significantly decrease food consumption and body weight gain in normal non-obese and genetically obese animals (Shaw et al., 1990). The magnitude of ß-FNA reduction of free feeding and body weight loss was consistent with that observed following the mu1 antagonist, naloxonazine,

both under acute (Simone et al., 1985), and chronic (Mann et al., 1988b) treatment. The pharmacological actions of naloxonazine and ß-FNA are indicative of mu-sensitive effects. Whereas naloxonazine blocked hyperphagia induced by the mu agonist, morphine, it failed to affect hyperphagia induced by the kappa agonists, ethylketocyclazocine and dynorphin, and the delta agonist, DADL (Mann et al., 1988a). Similarly, whereas ß-FNA significantly suppressed mu-sensitive DAMGO hyperphagia, it failed to alter the hyperphagia induced by the delta agonist, DSLET or the kappa agonist, U50,488H (Levine et al., 1991).

The stimulatory actions of ß-FNA upon intake parallel the temporal characteristics of ß-FNA as a reversible kappa agonist (Ward et al., 1982a). Such kappa agonists as ethylketocyclazocine, ketocyclazocine, tifluadom, bremazocine, U50,488H, dynorphin and MR2043 potently stimulate feeding (Jackson & Cooper, 1986; Lowy & Yim, 1983; Morley et al., 1983c; Morley et al., 1985; Gosnell et al., 1986b; Scott et al., 1984). In contrast, dynorphin and alpha-neo-endorphin antibodies attenuated food and water intake (Schulz et al., 1984). Central pretreatment with Nor-BNI was shown to block the hyperphagia induced by both kappa (U50,488H) and mu (DAMGO) agonists (Levine et al., 1990). Further, Nor-BNI pretreatment decreases feeding induced by electrical stimulation of the lateral hypothalamus (Carr et al., 1989). The present study demonstrated that pretreatment with Nor-BNI completely abolished the short-term (3-6h) hyperphagia induced by ß-FNA without affecting basal intake. In contrast, long-term (24h) pretreatment with ß-FNA failed to alter the short-term stimulation of intake of a second ß-FNA injection. Since the 24h interval between injections allowed for the blockade of mu receptors before the second ß-FNA administration, this demonstrates that the resultant hyperphagia is not dependent upon mu receptors for its expression. In conclusion, short-term ß-FNA treatment stimulates spontaneous feeding by

acting at kappa receptors while long-term β-FNA treatment suppresses free feeding by acting at mu receptors.

In collaboration with Dr. Gavril W. Pasternak, our laboratory confirmed the ability of central β-FNA to produce profound long-term inhibition of mu receptors in the striatum and hypothalamus. Pretreatment (24h) with β-FNA significantly reduced striatal mu opioid binding by 89% and hypothalamic opioid binding by 46%. These reductions were consistent with reductions in opioid binding following systemic administration of β-FNA (Portoghese et al., 1980; Takemori et al., 1981; Ward et al., 1982a,b), thus, indicating that β-FNA acts as an irreversible mu antagonist. The greater inhibition of mu binding in the striatum might illustrate the presumed concentration gradient following injection into the lateral ventricles and the proximity of the striatum to the injection site. The hypothalamic mu opioid binding following central β-FNA appears more functionally significant given the importance of this structure in the mediation of opioid-induced feeding by mapping studies (Gosnell et al., 1986b; Stanley et al., 1989). Since the medial (PVN and periventricular) hypothalamic nuclei appear to possess the greatest sensitivity to opioid ingestive effects, and are also more accessible to ventricular delivery of drugs, it would appear that these sites are excellent candidates for mediating the effects of centrally-administered β-FNA.

The role of the delta opioid receptor subtype in free feeding was also evaluated. Previous reports have implicated the delta opioid receptors in ingestive behavior. Central injection of DADL, DAME, DPDPE and DSLET enhanced consumption following central administration (Levine et al., 1986; McLean & Hoebel, 1983; Stanley et al., 1989; Tepperman & Hirst, 1983). The present study employed the delta selective antagonist, DALCE, to investigate further the role of the delta receptor in feeding. DALCE displays high affinity

for the delta receptors, moderate affinity for mu receptors and negligible affinity for the kappa receptors (Bowen et al., 1987), acting as a short-acting agonist at the delta receptor and secondarily at the mu receptor. Intracerebroventricular administration of DALCE significantly increased food intake after 2-10h only after the 10μg dose. This response is comparable with that observed for the above short-acting delta opioid receptor agonists (Gosnell et al., 1986a,b; McLean & Hoebel, 1983; Stanley et al., 1989; Tepperman & Hirst, 1983). While these and other agonists typically stimulate food intake for up to 4-6h, DALCE increases consumption over the 10 h time course. These data support the *in vitro* evidence of prolonged occupation of delta receptors by DALCE and suggest that DALCE may continue to act as an agonist at these receptors for some period of time. Interestingly, the short-term stimulatory effects were not monotonic, with DALCE lower (1μg) and higher (20 and 40μg) doses failing to alter food intake. The failure to observe consistent dose-response curves for the stimulatory effects of opiate agonists upon food intake has been quite common, beginning with initial characterizations of opioid hyperphagia with morphine (Sanger & McCarthy, 1980). Opioids not only enhance feeding, but also exert other behavioral effects, including sedation, hypoactivity and immobility. Therefore, the ability of higher doses of DALCE to stimulate food intake may have been compromised by subtle motor dysfunctions and/or slight sedation.

DALCE also produces a selective, long-lasting antagonism at the delta receptor. While pretreatment (24h) with DALCE failed to affect basal nociceptive thresholds, it nevertheless, blocked the analgesia produced by the delta-selective agonist, DPDPE, but not by mu (DAMGO) or kappa (U50,488H) agonists on the formalin and hot-plate tests (Calcagnetti et al., 1989a,b; Jiang et al., 1990). This DALCE antagonism was achieved at relatively low doses on

the hot plate (2μg) and formalin (6.7μg) tests. This is in direct contrast to the relatively high doses employed in the current study to examine ingestive behavior, which, needless to say, failed to alter intake. Central administration of DALCE (1-40μg) failed to reduce long-term food intake after 24, 48, 72 and 96h in freely feeding rats. However, it dose-dependently decreased body weight gain at 24h after injection. The highest DALCE dose (40μg) disrupted feeding efficiency 24h after central administration, as measured by the ratio of body weight to food intake. Central injection of the short-acting selective delta antagonist, ICI174,864 (1-100μg) produces dose- and time- dependent suppression in nocturnal free feeding, with the peak effects occurring at 1 and 2h after injection (Jackson & Sewell, 1985a). Similarly, ICI174,864 blocks DADL hyperphagia (Jackson & Sewell, 1985b). However, ICI174,864 administration also produces motor impairment (Long et al., 1988) which confounds its hypophagic properties, thus, presenting a problem in interpretation. Therefore, long-term responses in spontaneous ingestion appear not to be mediated by the delta opioid receptor subtype.

The present study demonstrated the ability of naltrexone to block the short-term hyperphagia following of DALCE over its full 10h time course, thus, providing further support that DALCE stimulates food consumption by its continued agonist actions on opioid receptors rather than some pharmacokinetic persistence of DALCE in the brain. Naltrexone is short-acting, losing its antagonist effects between 2 and 4h following administration. If DALCE were stimulating feeding by short-term occupancy of opioid receptors rather than some pharmacokinetic clearance in the brain, it would be expected that DALCE hyperphagia would return after the clearance of naltrexone. That naltrexone blocked the full duration of DALCE hyperphagia suggests that opioid antagonist pretreatment prevented DALCE from exerting its longer (10h)

stimulatory effects on intake by blocking initial attachment to the receptor site.

The particular receptor subtype that DALCE is stimulating to produce feeding is not consistent with the *in vitro* actions of DALCE, which indicates high affinity for delta receptors, moderate affinity for mu receptors and negligible affinity for kappa receptors (Bowen et al., 1987). Nor-BNI was most effective in blocking DALCE hyperphagia over its full 10h time course with the magnitude of antagonism ranging from 85-100%. In fact, Nor-BNI and naltrexone exhibited a similar profile of antagonism. On antinociceptive measures, pretreatment (24h) with DALCE has been shown to antagonize the acute agonist actions of a second DALCE dose (Jiang et al., 1990). However, prior DALCE pretreatment failed to significantly alter the hyperphagic effects of a second DALCE injection, with nonsignificant reductions of 28% and 38% occurring at 6 and 10h, respectively, after agonist treatment. β-FNA, the mu antagonist, also failed to significantly alter the hyperphagic effects of subsequent DALCE administration, with reductions of 39% and 26% respectively, noted 2 and 10h following agonist treatment. In effect then, the kappa antagonist, Nor-BNI, significantly blocked the hyperphagic response of DALCE, while β-FNA, a mu antagonist, and DALCE, a delta antagonist, failed to significantly alter the hyperphagic effects of a second DALCE injection. Several explanations may be provided to comprehend DALCE's variable responses in ingestive behavior. DALCE forms covalent attachments to the delta, but not the mu or kappa, receptors, thus, producing irreversible antagonism of this receptor. It acts as a short-acting agonist at the delta and secondarily at the mu receptors (Bowen et al., 1987). Thus, the inability of long-term pretreatment with DALCE to block the short-term hyperphagia induced by DALCE might indicate that while the short-term delta agonist actions of DALCE occurred, the expected long-term

delta antagonist actions of DALCE did not. Perhaps DALCE stimulated the consumption of food directly through weak kappa agonist effects and not through its delta or mu actions. This possibility would support the contention that kappa receptors are the integral subtype responsible for the opioid modulation of feeding (Gosnell et al., 1986a; Morley et al., 1982a; Morley et al., 1983a,c; Morley & Levine, 1981). Alternatively, DALCE may have stimulated food intake directing through delta and mu receptors which, in turn, require an intact kappa receptor-mediated system for its expression. Thus, the relative inability of either delta antagonism by DALCE alone or mu antagonism by β-FNA alone to block DALCE hyperphagia would be explained by the ability of DALCE to stimulate the remaining receptor subtype. In fact, it has been shown that different opioid receptor subtype agonists of the mu, kappa and delta receptors increase food intake (Morley et al., 1983a). Nor-BNI blocked DALCE hyperphagia over the 10h time course. This short-acting antagonist was shown to suppress the hyperphagic response of the kappa selective agonist, U50,488H, the mu selective agonist, DAMGO, as well as the delta selective agonist, DSLET. Thus, it was concluded that the kappa opioid receptor may form part of the final common pathway for opioid-induced feeding (Levine et al., 1990).

B. Nocturnal Intake

During the night, rats eat larger meals and with greater frequency (Le Magnen, 1981). Dynorphin levels have been shown to be elevated in the hypothalamus during the active cycle of the rat, suggesting a role of the kappa opioid receptor subtype in this form of ingestion (Przewlocki et al., 1983). Naloxone and naltrexone were shown to significantly decreased nocturnal intake (Brands et al., 1979; Cooper, 1980; Jalowiec et al., 1981; Mandenoff et

al., 1984). The current study further demonstrates that naltrexone suppresses early dark feeding by 60%. Similarly, pretreatment with Nor-BNI significantly decreased nocturnal feeding for up to 12h following the onset of the dark cycle despite the short-acting nature of this kappa antagonist (Takemori et al., 1988). The delta selective antagonist, ICI174,864, transiently reduced nighttime feeding with the peak effects observed 1 and 2h after injection. However, such a response was associated with motor dysfunction and paraplegia following intrathecal administration (Jackson & Sewell, 1985a; Long et al., 1988). An evaluation of the roles for mu and delta receptors in nocturnal intake occurred in the present free feeding experiments involving ß-FNA (Exp. 1A) and DALCE (3A). In both of these free-feeding paradigms, all rats received microinjections at the beginning of the light cycle, and subsequent intake measures were taken over the light cycle, including at the end (12h) of the light cycle. Another intake measure was taken at the end (24h) of the dark cycle, and the difference between 12 and 24h intake represents nocturnal intake. Whereas ß-FNA failed to alter food intake after 12h (light cycle), it significantly reduced intake after 24h, an effect that had to be due to reductions in nocturnal intake. This indicates a role for the mu reception in nocturnal free feeding. In contrast, DALCE failed to exert effects at either interval, suggesting that the delta receptor is not directly involved in nocturnal intake.

Naltrexone, Nor-BNI and ß-FNA dose-dependently inhibit free feeding at relatively low doses. DALCE, on the other hand, failed to exert a monotonic response, indicating that its binding to the delta receptors is not effective enough to inhibit feeding. It appears that free feeding is modulated not by a single receptor subtype, but by a number of receptors, displaying a rank order response in terms of magnitude, in this case, with the general (naltrexone) and

kappa (Nor-BNI) receptors producing the greatest inhibition in free feeding. The mu (ß-FNA) receptors are also activated and its response are more potent than the delta receptors, which is the least effective (general=kappa>mu>>delta). This differential response indicates that some antagonists may be more effective in inhibiting intake. However, since the degree of binding to a receptor varies, an antagonist's "selective" response limits a definitive interpretation.

C. Deprivation-induced Feeding

Central ß-FNA significantly attenuated deprivation-induced feeding by 30-33% following the 10µg dose and by 43-49% following the 20µg dose. Levine and co-workers similarly demonstrated that ß-FNA, administered in the PVN 24h before the reintroduction of food to deprived animals, reduced the compensatory increase in food intake by 24% (Levine et al., 1989). The greater decreases observed following intracerebroventricular administration as compared to PVN treatment may reflect the possibility that additional hypothalamic and extra-hypothalamic sites may be involved in the mu-mediated opioid modulation of deprivation-induced feeding. Morphine was shown to stimulate feeding following injection in the PVN (McLean & Hoebel, 1983) and the ventromedial hypothalamus (Tepperman et al., 1980; Thornhill & Saunders, 1984), and these effects were antagonized by naloxone. The degree of inhibition following naloxone and naltrexone treatment in food deprivation was consistent with that observed with central ß-FNA (Brands et al., 1979; Brown & Holtzman, 1979; Holtzman, 1974). Pretreatment (24h) with naloxonazine, a mu1 antagonist, also significantly reduced deprivation-induced feeding (Simone et al., 1985), indicating that the mu opioid receptor subtype and its high-affinity binding site mediate intake induced by food deprivation. However,

deprivation-induced feeding appears to be modulated by the kappa opioid receptor as well. A decrement in dynorphin levels in the hypothalamus and striatum (Vaswani & Tejwani, 1986), as well as the cortex (Majeed et al., 1989; Morley et al., 1982b) was observed following food deprivation. Central administration of the short-term kappa antagonist, Nor-BNI, attenuated feeding by 28% after food deprivation (Levine et al., 1990). The weaker kappa antagonist, MR2266, was also shown to decrease consumption in rats deprived of food (Sanger et al., 1983), producing further evidence for a kappa involvement in ingestion. The delta opioid receptor plays an extremely minimal role in deprivation-induced feeding. Long-term DALCE pretreatment (24h) failed to alter short-term (2-4h) deprivation-induced feeding and only sporadically decreased long-term (24-72h) intake after deprivation. The highest DALCE dose (40μg) significantly retarded body weight recovery after 24h in deprived rats while the lower doses (10 and 20μg) stimulated body weight recovery after 48 and 72h in deprived rats. Essentially then, deprivation-induced feeding is modulated by all of the opioid receptor subtypes, with the mu receptors being more potent. The kappa receptors, in turn, exert a more effective response than the delta receptors (general≈mu>kappa>>delta).

D. Glucoprivic Feeding

Glucoprivation is induced by 2DG and insulin, however, their hyperphagic responses are dissociated based on their mechanism of action. Central and systemic naloxone and naltrexone attenuated 2DG hyperphagia with greater effectiveness than insulin-induced feeding (Lowy et al., 1980; Ostrowski et al., 1981; Yim et al., 1982). These general antagonists either failed to alter (Lowy et al., 1980) or marginally reduced feeding induced by insulin (Beczkowska &

Bodnar, 1991; Levine & Morley, 1981; Ostrowski et al., 1981; Rowland & Bartness, 1982). The data indicate that intracerebroventricular administration of β-FNA completely abolished 2DG hyperphagia, suggesting mu-mediated activity. This is in contrast to the inability of the irreversible mu_1 antagonist, naloxonazine, to affect the intake induced by 2DG (Simone at al., 1985). The ability of β-FNA ($mu=mu_1+mu_2$) and inability of naloxonazine (mu_1) suggests that the low-affinity mu_2 opioid receptor subtype is in part responsible for this form of glucoprivic feeding. Nor-BNI potently and dose-dependently reduced 2DG feeding, providing evidence for kappa opioid receptor involvement. However, the delta opioid receptor subtype appeared not to modulate 2DG glucoprivation. 2DG hyperphagia was unaffected by long-term pretreatment (24h) with DALCE or ICI174,864 (Jackson & Sewell, 1986b). Recent data from our laboratory (Beczkowska & Bodnar, 1991) indicate that opioid control of glucoprivic intake dissociates for 2DG and insulin. A significant reduction in insulin-induced feeding was demonstrated following the mu antagonist, β-FNA, but not following either the kappa antagonist, Nor-BNI, the delta antagonist, DALCE or the mu_1 antagonist, naloxonazine. Thus, whereas mu_2 and kappa receptors mediate 2DG hyperphagia, only mu_2 receptors participate in the smaller opioid mediation of insulin hyperphagia.

E. Palatable Intake

Highly palatable diets are modulated by the endogenous opioid system. The mu opioid receptor subtype has been implicated in palatability. Morphine and DAMGO were shown to enhance intake of highly palatable solutions (Belluzzi & Stein, 1982; Gosnell & Majchrzak, 1990). Central and peripheral naloxone and naltrexone suppress the preference and intake of palatable diets and solutions (Apfelbaum and Mandenoff, 1981; Cooper & Gilbert, 1984;

Cooper et al., 1985a,b; Islam & Bodnar, 1990; Marks-Kaufman & Kanarek, 1981). Whereas intracerebroventricular administration of β-FNA reduced the intake of a high-fat diet by 37%, the mu$_1$ antagonist, naloxonazine failed to affect consumption, again, arguing for a role of the mu$_2$ opioid receptor subtype in this response as well (Islam & Bodnar, 1990). However, the kappa opioid receptor subtype has also been implicated in the consumption of highly palatable diets (Cooper et al., 1985b). The kappa agonists, U50,488H, tifluadom, ethylketocyclazocine and bremazocine, stimulate the intake of a wet mash diet consisting of sweetened condensed milk, tap water and powered chow (Jackson & Cooper, 1985). In contrast, Nor-BNI and the putative kappa antagonist, MR2266, were shown to decrease intake of sweetened solutions (Cooper et al., 1985a; Calcagnetti et al., 1990). The present study indicated that central pretreatment with Nor-BNI potently and dose-dependently reduced consumption by 79% following exposure to a high-fat diet. Nor-BNI significantly inhibited intake of the diet, even at a very low dose (1μg), further supporting the role of the kappa opioid receptor in palatability. Additionally, the role of delta opioid receptor subtype was evaluated. While such delta agonists as DTLET and DPDPE, significantly increased intake of palatable solutions (Gosnell et al., 1990; Gosnell & Majchrzak, 1990), the delta selective antagonist, ICI174,864, failed to alter the hyperphagia induced by exposure of a high-fat diet at doses that failed to produce motor dysfunction (Islam & Bodnar, 1990). The current observation indicated that DALCE pretreatment 24h prior to the introduction of a high-fat diet failed to significantly alter the increased consumption. In fact, the lower DALCE (1μg) dose significantly stimulated intake at both 1 and 2h following the introduction of the diet. Therefore, the kappa and mu$_2$ receptors, but not the mu$_1$ and delta receptors, are more effective in stimulating palatable consumption

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(kappa>general>mu2>mu1*delta).
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Conclusions

The existence of distinct opioid receptor subtypes, namely mu, kappa and delta receptors, are indicative of functional significance (Lord et al., 1977; Martin et al., 1976; Pasternak and Wood, 1986). The mu receptors have been implicated in supraspinal analgesia, respiratory depression, inhibition of gastrointestinal transit and some forms of physical dependence. The kappa receptor mediates spinal analgesia and sedation while the delta receptor has been implicated in both spinal and supraspinal analgesia, growth hormone release and dopamine turnover. These different opioid receptor subtypes are also implicated in the control of ingestive behaviors, with opioid receptor agonists typically stimulating food intake under a variety of situations. Morphine facilitates consumption following peripheral (Morley et al., 1983a; Thornhill and Saunders, 1983) as well as central (McLean and Hoebel, 1983; Tepperman et al., 1980; Thornhill and Saunders, 1984) administration. In both free-feeding and mildly-deprived (4-5 hours) rats, morphine stimulates food and water intake, but decreases intake in rats deprived of food for a period of 24h (Kumar et al., 1971; Sanger & McCarthy, 1980, 1981; Jalowiec et al., 1981). Central administration of DAMGO, a selective mu agonist, was also shown to increase food consumption (Gosnell et al., 1986a). Similarly, agonists of the kappa and delta receptors enhanced food intake following central injection (Gosnell et al., 1986a; Morley et al., 1983a; Tepperman & Hirst, 1983).

In contrast, opiate receptor antagonists attenuate feeding, particularly under spontaneous intake, deprivation-induced feeding, glucoprivic feeding, palatable intake and nocturnal feeding. Under such diverse ingestive situations, where changes in the levels of endogenous opioids are evidenced, a

single or a combination of opiate receptor subtype population may mediate feeding. The general opiate receptor antagonists, naloxone, naltrexone and ß-CNA exhibit a suppressive response in ingestion regardless of the paradigm employed. ß-FNA facilitated short-term consumption by acting through the kappa opioid receptor while reducing long-term feeding and body weight gain through the mu opioid receptors. Antagonists of the kappa opioid receptor, as Nor-BNI and MR2266, attenuated food intake, whereas antagonists of the delta opioid receptors, as DALCE and ICI174,864, failed to alter intake under spontaneous and food deprived conditions. It appears then, that free feeding, deprivation-induced intake and nocturnal feeding are mediated by the mu and kappa, but not the delta, opioid receptor subtypes. Glucoprivation, induced by 2DG, and intake of highly palatable diets were blocked by naloxone and naltrexone (Apfelbaum & Mandenoff, 1981; Cooper & Gilbert, 1984; Cooper et al., 1985a,b; Lowy et al., 1980; Marks-Kaufman & Kanarek, 1981; Ostrowski et al., 1981; Yim et al., 1982). Similarly, ß-FNA (Islam & Bodnar, 1990) and Nor-BNI attenuated the hyperphagia induced by 2DG and a high-fat diet. However, naloxonazine, the mu_1 antagonist, and DALCE and ICI174,864, the delta antagonists, failed to alter feeding induced by 2DG or of a high-fat diet (Jackson & Sewell, 1985b; Simone at al., 1985). Therefore, both glucoprivation and palatability are mediated by the mu_2 and kappa, but not the mu_1 or delta, opioid receptor subtypes in the brain.

While ß-FNA, Nor-BNI and DALCE selectively bind to the mu, kappa and delta receptors respectively such selective response should be viewed with caution. Some drugs like ß-FNA and Nor-BNI, are more selective than others, like DALCE, indicating that selectivity is not a definitive phenomenon. DALCE, unlike ß-FNA and Nor-BNI, failed to produce a dose response effect suggesting that this antagonist may bind to other receptors and not solely to the delta

receptors. Thus, the results obtained with DALCE may not be a delta-selective effect. Further, different feeding models were employed to provide an integrative explanation of ingestion. Depending on the feeding model, an antagonist may be more effective in blocking consumption, such as β-FNA, which was rather effective in inhibiting free feeding, deprivation-induced feeding and glucoprivation. Nor-BNI, on the other hand, was quite effective in inhibiting high-fat intake as well as nocturnal feeding, but displayed smaller inhibitory effects upon deprivation-induced feeding (Levine et al., 1990). Thus, the rank order response obtained as a result of the specific antagonists is indicative of the fact that a number of receptors are activated and that selectivity should not be viewed as absolute.

Essentially then, while multiple opiate receptor populations are involved in the regulation of ingestive behavior, distinct opioid receptor subtypes are activated and exhibited differential responses based on the internal state of the animal. As mentioned, the mu, kappa and delta opioid receptors facilitate consumption following central administration. The kappa, as opposed to mu and delta, opiate agonists are more potent in stimulating feeding, thus indicating that the kappa opioid receptors play an integral role in the initiation of feeding (Morley et al., 1985). The short-acting kappa antagonist, Nor-BNI, blocked the hyperphagic responses induced by the mu (DAMGO), kappa (U50,488H) and delta (DSLET) agonists (Levine et al., 1990). In addition, short-term β-FNA and DALCE hyperphagia were also reduced by Nor-BNI, suggesting that the kappa receptor is the final common pathway of ingestion.

Further evidence that the opioid receptor subtypes display differential responses in feeding is based on their site of action in the central nervous system. Opiate receptors are distributed throughout the central and peripheral nervous systems (Akil et al., 1984; Atweh & Kuhar, 1977; Mansour et

al., 1987). Such areas which have been implicated in the modulation of opioid-induced hyperphagia include the medial hypothalamus, specifically the PVN and the periventricular areas, nucleus accumbens, amygdala, striatum, globus pallidus, the ventromedial hypothalamus, the lateral hypothalamus and the gastrointestinal tract (Gosnell et al., 1986b; Morley, 1987; Stanley et al., 1989). Naloxone significantly reduced food intake following injection in the PVN, the ventromedial hypothalamus and the globus pallidus, but not the lateral hypothalamus or the striatum (Gosnell et al., 1986b). Indeed, β-FNA decreased mu binding in the striatum and hypothalamus, with a greater reduction occurring in the striatum. β-FNA also attenuated deprivation-induced intake following injection in the PVN (Levine et al., 1989). The kappa opioid receptors are concentrated in areas as the nucleus tractus solitaris, parabrachial nucleus, thalamus, medial nuclei of the amygdala and medial hypothalamus, which are involved in the regulation of food and fluid intake, as well as taste perception (Lynch et al., 1985). Nor-BNI, injected in the lateral ventricles or the PVN, significantly decreased feeding induced by either food deprivation or opioid agonists (Levine et al., 1989, 1990). Similarly, Nor-BNI antagonized the feeding induced by electrical stimulation of the lateral hypothalamus (Carr et al., 1989). Additionally, the delta opioid receptors are densest in areas as the nucleus accumbens and amygdala (Mansour et al., 1987). Agonists of the delta opioid receptor stimulate feeding following injection in the ventromedial hypothalamus, PVN, perifornical hypothalamus and amygdala (McLean & Hoebel, 1983; Stanley et al., 1989; Tepperman & Hirst, 1983). Thus, the mu, kappa and delta opioid receptor subtypes are concentrated in different areas of the central nervous system which may contribute to their dissimilar responses in the various feeding situations. However, while such diverse areas in the brain mediate ingestion, the primary

sites of endogenous opioid actions in appetite control are the hypothalamus and brain stem structures. It appears that the opioids in these sites act in unison to regulate the physiological processes involved in maintaining an adequate nutritional state. The hypothalamus may be the site at which such integration occurs, thereby regulating ingestion and maintaining homeostasis.

Ingestive behavior is a complex and integrative process, involving not only the endogenous opioids, but also a number of neuropeptides and neurotransmitters. Cholecystokinin, bombesin, neurotension and thyrotropin-releasing hormone among others decrease food intake, while neuropeptide Y, peptide YY, galanin and opioid peptides among others stimulate feeding (see reviews: Leibowitz, 1987; Morley, 1987). Opioid peptides appear to be closely linked to the classical neurotransmitters, namely norepinephrine, epinephrine and serotonin, interacting to control ingestive behavior. Whereas hypothalamic stimulation with norepinephrine and epinephrine elicit feeding in satiated animals, such stimulation with serotonin exhibits a suppressive effect on food intake in freely feeding or food deprived animals. In fact, norepinephrine and serotonin act in an antagonistic manner to control carbohydrate and protein ingestion at the beginning of the animal's active cycle. Norepinephrine stimulates the first meal of the nocturnal period which is generally rich in carbohydrate. As a result of this meal, there is a surge of serotonin activity which appears to activate the satiety mechanism, terminating the carbohydrate meal, and subsequently, switching the animal's preference toward protein, the macronutrient of choice for the second meal of the nocturnal cycle. The alpha2 noradrenergic system and the opiate receptor system were shown to induce carbohydrate and protein meals, respectively, in the early hours of the dark cycle (Shor Posner et al., 1986). Opioid stimulation of ingestion was shown to be blocked by alpha2 noradrenergic antagonists.

Opiate peptides decrease neuronal firing of the PVN, as well as norepinephrine-induced feeding. Adrenalectomy attenuated morphine-induced feeding, but abolished norepinphrine-induced feeding. Opiates and the alpha2 noradrenergic receptors appear to function in a sequential manner to regulate ingestion, suggesting that the opiates exert their responses through the alpha2 noradrenergic synapse. Thus, opioids are not single entities working to stimulate consumption, but rather, work in unison with other systems to maintain ingestive homeostasis.

In addition to the fact that opioid receptor subtypes do not exert similar actions in feeding, this dissertation also addressed the issue regarding antagonists' differential responses based on the animal's metabolic state. While the general opiate receptor antagonists suppressed feeding under normal and aberrant circumstances, the specific opiate antagonists yielded selective effects. The mu and kappa, but not the delta, opioid receptor antagonists attenuated normal feeding, feeding induced by regulatory challenges and palatable intake. Differential responses may due to the pharmacological profile of the drug and/or to their site of action, be it at the individual opioid receptor subtype population or at different brain sites. Again, the selectivity of a drug also raises some questions since a drug may bind to a number of receptor sites, but with different affinity. For example, DALCE transiently stimulates short-term food intake through the delta receptor. However, DALCE also displays an affinity for the mu receptor, thus the short-term hyperphagia may due to activation of the mu rather than the delta receptor. Further, DALCE's hyperphagic effect may occur through a weak kappa agonist response as the kappa receptors play an integral role in feeding (Gosnell et al., 1986a). This is contrary to the biochemical evidence indicating that DALCE has negligible affinity for the kappa receptor. Therefore, while an

antagonist is labelled as selective, it should be viewed with skepticism. It is also important to recognize that a drug's inhibitory effect may be the result of aversion or malaise. However, while such possibility should be entertained, it would be erroneous to conclude that the antagonists employed in this study produced such responses since the drugs were centrally administered at relatively low doses and since the animals exhibited similar behaviors following each injection under diverse conditions. In conclusion, under normal feeding, regulatory challenging situations and palatable conditions, antagonists differentially respond to food intake.

In addition to regulating food intake, endogenous opioids also control fluid consumption and are involved in the sensory and nutrient aspect of feeding. For example, morphine facilitates fat consumption while decreasing carbohydrate intake in rats maintained on a self-selection diet (Marks-Kaufman & Kanarek, 1980). However, in food deprived animals, morphine enhances protein intake (Shor-Posner et al., 1986). Naloxone decreases fat, and to a lesser extent, carbohydrate intake in rats when placed on a self-selection diet (Marks-Kaufman & Kanarek, 1981). While ß-FNA and Nor-BNI reduced spontaneous intake, palatable intake and deprivation-induced feeding, it would be interesting to determine which particular macronutrient were altered by these different antagonists. Further, opioids enhanced the taste of sweet and nonsweet solutions. Central administration of morphine stimulates the consumption of saccharin and sucrose solutions (Calcagnetti & Reid, 1983; Cooper, 1983; Czirr & Reid, 1989), as well as sweetened milk (Belluzzi & Stein, 1982). DAMGO and DPDPE were also shown to increase intake of hypertonic (0.6%) saline solutions (Gosnell et al., 1990; Gosnell & Majchrzak, 1990). Naloxone and the kappa antagonists, Nor-BNI and MR2266, reduced intake of palatable solutions (Calcagnetti et al., 1990; Cooper et al., 1985a,b). Future

investigation may decipher the role of the mu and delta opioid receptors' involvement in fluid consumption and palatable intake. Additionally, lesion studies may also be conducted to determine which particular site modulate ingestion, and at the same time, identifying the opioid receptor subtypes which are involved. Agonists enhance while antagonists inhibit food intake following injection in the PVN (Gosnell et al., 1986b). Lesion placed in this area was shown to increase food intake and body weight gain (Shor-Posner et al., 1986). PVN lesions significantly attenuated morphine induced feeding whereas ventromedial hypothalamic lesions failed to alter naloxone anorexia (King et al., 1979). Therefore, lesion studies (in the PVN or the ventromedial hypothalamic areas) may facilitate an understanding of the role of the mu, kappa and delta opioid receptor subtypes in ingestion with the assistance of the selective opioid receptor antagonists.

Glossary

Name of Drug	Pharmacological Action
Beta-chlornaltrexamine (ß-CNA)	general opiate antagonist
Beta-endorphin	epsilon, mu, delta agonist
Beta-funaltrexamine (ß-FNA)	short-term kappa agonist
	long-term mu antagonist
Bremazocine	kappa agonist
Butorphanol tartrate	kappa agonist
[D-Ala^2,D-Leu^5]-Enkephalin (DADL)	delta agonist
[D-Ala^2,Leu^5,Cys^6]-Enkephalin (DALCE)	delta antagonist
[D-Ala^2,MePhe^4,Gly-ol^5]-Enkephalin (DAMGO)	mu agonist
2-deoxy-D-glucose (2DG)	antimetabolic analog
[D-Pen^2,D-Pen^5]-Enkephalin (DPDPE)	delta agonist
[D-Ser^2,Leu^5]-Enkephalin-Thr^6 (DSLET)	delta agonist
Dynorphin	kappa agonist
Ethylketocyclazocine (EKC)	kappa agonist
ICI174,864	delta antagonist
Ketocyclazocine	kappa agonist
M80	delta antagonist
Morphine	mu agonist
MR2043	kappa agonist
MR2266	putative kappa antagonist
N-allylnorphenazocine (SKF-10,047)	sigma receptor agonist
Naloxonazine	mu1 antagonist

Naloxone	general opiate antagonist
Naltrexone (NTX)	general opiate antagonist
Nor-binaltorphimine (Nor-BNI)	kappa antagonist
Tifluadom	kappa agonist
U50,488H	kappa agonist

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