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Differential effects of opioid receptor subtype antagonists upon fluid intake of sucrose, saccharin and carbohydrate maltose dextrin solutions in rats

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DIFFERENTIAL EFFECTS OF OPIOID RECEPTOR SUBTYPE ANTAGONISTS UPON FLUID INTAKE OF SUCROSE, SACCHARIN AND CARBOHYDRATE MALTOSE DEXTRIN SOLUTIONS IN RATS.

by

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Abstract

Differential Effects of Opioid Receptor Subtype Antagonists Upon Fluid Intake of Sucrose, Saccharin And Carbohydrate Maltose Dextrin Solutions In Rats

By

Iwona W. Beczkowska

Advisor: Dr. Richard J. Bodnar

The general non-specific opioid antagonists appear more potent in inhibiting intake of palatable foods, suggesting that opiate antagonist-induced inhibition of ingestive behavior might be due to its effects upon ingestive hedonics. The aim of this dissertation was to evaluate the role of specific opioid receptor subtypes in modulating cumulative (5 min intervals) intake over 1 h of palatable solutions following central administration of general (naltrexone: 1, 5, 20 and 50 ug) and specific mu (beta-funaltrexamine, B-FNA: 1, 5 and 20 ug), mu_1 (naloxonazine: 10, 20 and 50 ug), kappa (nor-binaltorphamine, Nor-BNI: 1, 5 and 20 ug), $delta_2$ (naltrindole: 1, 5 and 20 ug) and $delta_1$ (D-Ala^2, Leu^5, Cys^6-enkephalin, DALCE: 10, 20 and 40 ug) opioid receptor antagonists. Antagonists were examined for their ability to alter intake of the sucrose (10%), which has both palatable and nutritive properties, and saccharin (0.1%) which is palatable but non-nutritive. In addition, intake of carbohydrate maltose dextrin (CMD, 10%) was evaluated to determine whether opioid modulation of palatable intake extends to polysaccharide solutions. Finally, to determine whether any opioid antagonist effects upon sucrose, saccharin and CMD intake are specific to their presumed palatable qualities or due to reductions of fluid intake per se, the effects of general and specific opioid receptor subtype antagonists upon deprivation-induced water were studies. Deprivation-induced water intake was significantly reduced by central

administration of naltrexone (65%) and B-FNA (40%), but not by naloxonazine or any of the other antagonists suggesting a mu_2 mediation of this form of intake. Sucrose intake was significantly inhibited by naltrexone (65%), B-FNA (35%) and Nor-BNI (55%), suggesting a primary kappa role and secondary mu_2 role in this form of intake. Saccharin intake was significantly reduced by naltrexone (61%) and by naltrindole (75-90%), suggesting a $delta_2$ mediation of this form of intake. Thus, nutritional consequences of an ingestate appear to be important determinants of which of the opioid receptor subtypes modulates a given response. CMD intake was significantly inhibited by naltrexone (68%) indicating opioid involvement in polysaccharide intake. B-FNA (46%), but not naloxonazine or any other antagonist reduced CMD intake suggesting mu_2 mediation. Regression analysis revealed rank-order potencies (ID_{50}) of opioid antagonists. Water intake: Nor-BNI (50 mM) > naltrexone (90 mM) > > B-FNA (110 mM) > DALCE (180 mM) > naltrindole (260 mM) > naloxonazine (280 mM). Sucrose intake: Nor-BNI (20 mM) > naltrexone (50 mM) > naltrindole (60 mM) > DALCE (100 mM) > naloxonazine (120 mM) > B-FNA (450 mM). Saccharin intake: naltrindole (30 mM) > Nor-BNI (40 mM) > naltrexone (70 mM) > DALCE (100 mM) > naloxonazine (280 mM) > B-FNA (470 mM). CMD intake: naltrexone (30 mM) > Nor-BNI (40 mN) > naltrindole (90 mM > naloxonazine (160 mM) > DALCE (290 mM) > B-FNA (660 mM). Thus the mu_2 subtype, alters intake with low potency and to a limited degree, suggesting marginal involvement in modulation of palatable intake. The kappa opioid receptor appears primarily important in the mediation of intake of ingestates with both palatable and post-ingestive consequences. In contrast, the delta opioid receptor appears to be involved in the mediation of sweet-tasting but non-nutritive intake.

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This dissertation is dedicated in memory of my grandmother, Genowefa, whose love and devotion I will never forget.

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CHAPTER I. INTRODUCTION

The endogenous opioid system has been implicated in ingestive behavior with opioid agonists typically stimulating intake, and general opioid antagonists inhibiting intake. In this regard, the general opioid antagonists, naloxone and naltrexone, inhibit spontaneous and deprivation-induced food and water intake, as well as intake induced by glucoprivation, stress, and diabetes. In addition, naloxone and naltrexone appear to be more potent in inhibiting intake of palatable foods, suggesting that opiate antagonist-induced inhibition of ingestive behavior may be due to its effects upon ingestive hedonics. The existence of multiple opioid receptor subtypes led to the development of highly-selective opioid receptor subtype agonists and antagonists. Studies employing selective antagonists for mu (beta-funaltrexamine, B-FNA), mu_1 (naloxonazine), kappa (nor-binaltorphamine, Nor-BNI), $delta_2$ (naltrindole), and $delta_1$ (D-Ala^2, Leu^5, Cys^6-enkephalin, DALCE) opioid receptor subtypes found differential involvement of opioid receptor subtypes in different feeding situations. Thus, free feeding and deprivation-induced feeding were significantly reduced by mu, mu_1 and kappa antagonists. Hyperphagia induced by 2-deoxy-D-glucose was reduced by mu and kappa antagonists but insulin-induced hyperphagia was attenuated by only mu antagonists. Finally, hyperphagia following exposure to a palatable, high-fat diet was significantly reduced by kappa and mu antagonists, suggesting a role for these two receptor subtypes in the regulation of intake of palatable ingestates.

Intakes of highly-palatable sucrose and saccharin solutions are significantly

inhibited by nonselective opiate antagonists with the reductions occurring gradually during presentation of the solutions. These data suggest that opioid antagonism interferes with the maintenance not the initiation of palatable intake. The aim of this dissertation is to evaluate the role of specific opioid receptor subtypes in modulating intake of palatable liquid ingestates following central administration of general (naltrexone) and specific mu (B-FNA), mu_1 (naloxonazine), kappa (Nor-BNI), $delta_2$ (naltrindole) and $delta_1$ (DALCE) opioid receptor antagonists. Antagonists are examined for their ability to alter intake of a sugar, sucrose, which has both palatable and nutritive properties, and saccharin which has palatable, but non-nutritive properties. In addition, intake of carbohydrate maltose dextrin (CMD) is evaluated to determine whether opioid modulation of palatable intake extends to this polysaccharide. Finally, to determine whether any opioid antagonist effects upon sucrose, saccharin and CMD intake are specific to their palatable qualities or due to reductions of fluid intake per se, the effects of general and specific opioid receptor subtype antagonists upon deprivation-induced water intake are studied. The following sections provide background information pertaining to: I) opioid peptides, II) opioid receptor subtypes, III) opioid receptor interactions with opioid ligands, IV) opioids in feeding behavior, and V) a rationale for the present experiments.

I. Opioid Peptides.

Opioid peptides are derived from one of three precursor molecules: proopiomelanocortin (POMC), proenkephalin, and prodynorphin, but share a

common opiate-active core: Tyr-Gly-Gly-Phe (Sherman, Akil and Watson, 1989).

1. Proopiomelanocortin-derived products. The carboxy terminus of POMC contains beta-endorphin and its precursor beta-lipotropin (beta-LPH). The mid-region of beta-LPH contains ACTH-(1-39), which in turn can be cleaved into alpha-MSH and Corticotropin-Like Intermediate Lobe Peptide (CLIP) (Mains, Eipper and Ling, 1977). The amino terminus of beta-LPH is gamma-MSH (Roberts, Seeburg, Shine, Herbert, Baxter and Goodman, 1979). Processing and post-translation events are best understood in pituitary POMC (Zakarian and Smyth, 1982). POMC cells in the anterior and intermediate lobes of the pituitary process the precursor quite differently. In general, the intermediate lobe produces smaller products, which have undergone more post-translational modifications, than does the anterior lobe. Similar differences between the intermediate and anterior lobes are seen in the processing of ACTH (Ling, Ying, Minick and Guillamin, 1979). Biochemical studies in rat indicated that the POMC precursor yields ACTH and beta-endorphin in the anterior lobe of the pituitary, but alpha-MSH and beta-endorphin in the intermediate lobe (Khachaturian, Lewis, Schafer and Watson, 1985).

In the brain, there are two distinct cell groups which contain POMC-derived peptides (Khachaturian et al., 1985). The first group is located in the arcuate nucleus of the hypothalamus with some cells scattered along the periarcuate medial-basal hypothalamus. Beta-endorphin, beta-lipotropin, and ACTH immunoreactivities are co-localized in the same arcuate neurons (Watson, Akil, Richard and Barchas,

1978). The second group is found in the caudal parts of the nucleus tractus solitarius (NTS), which is a group of small neurons that reside within the commissural nucleus and which project laterally to the lateral reticular nucleus. POMC neurons in the arcuate nucleus project extensively throughout the brain (Khachaturian et al., 1985). Rostrally-projecting fibers course through periventricular diencephalic and telencephalic areas, innervating many hypothalamic and limbic structures, including the preoptic area, septum, and the bed nucleus of stria terminalis. Lateral projections of arcuate POMC neurons extend through the medial-basal hypothalamic region and the temporal cortex. Dorso-caudally projecting fibers course through the dorsal diencephalon to enter the mesencephalon and brainstem, innervating many areas associated with nociceptive, gustatory, and other sensory integration. These areas include the periventricular thalamus and periaqueductal gray. Other caudal projections enter the brainstem ventrally to innervate numerous areas of the reticular formation, such as nucleus reticularis gigantocellularis, reticularis lateralis, and medullary raphe nuclei. Further brainstem sites containing POMC-derived peptides include nuclei parabrachialis and ambiguus, NTS, and the dorsal motor nucleus of vagus.

2. Proenkephalin-derived products. Proenkephalin contains the coding for several active opioid peptides including leu-enkephalin, met-enkephalin, two extended forms of met-enkephalin (met-enkephalin-Arg-Phe and met-enkephalin-Arg-Gly-Leu) and several other large opioid molecules (Comb, Herbert and Crea, 1982).

Proenkephalin is synthesized in many neuronal systems throughout the CNS. Initial immunocytochemical studies (Elde, Hokfelt, Johansson and Terenius, 1976) demonstrated a very similar distribution pattern for both leu- and met-enkephalin which was dissimilar to the distribution of beta-endorphin and other POMC-related peptides. Immunoreactive enkephalin perikarya have been noted in most regions of the telencephalon, including the cerebral cortex, olfactory tubercle, amygdala, hippocampus, bed nucleus of the stria terminalis and preoptic area. In the diencephalon, enkephalin perikarya are seen in most hypothalamic nuclei, and in the periventricular and lateral geniculate nuclei of thalamus. In the midbrain, proenkephalin cells are localized in the colliculi, periaqueductal gray, and interpeduncular nucleus. In the pons and medulla, perikarya are seen in the parabrachial, dorsal tegmental, vestibular and raphe nuclei, nucleus reticularis gigantocellularis and paragigantocellularis, NTS, lateral reticular nucleus, spinal trigeminal nucleus and the dorsal horn of the spinal cord (e.g., Hokfelt, Elde, Johansson, Terenius and Stein, 1977; Khachaturian, Lewis, Hollt and Watson, 1983; Sar, Stumpf, Miller and Chang, 1978). In addition to the areas containing both cell bodies and fibers, many other brain regions show varying densities of fiber and terminal-like enkephalin immunoreactivity. Most proenkephalin fibers are interneurons intrinsic to the particular area.

3. Prodynorphin-derived products. Prodynorphin is cleaved to produce three leu-enkephalin containing peptides: alpha and beta-neo-endorphin (Kangawa,

Minamino, Chino, Sakakibara and Matsuo, 1981), dynorphin A, and dynorphin B (Goldstein, Fischli, Lowney, Hunkapiller, and Hood, 1981). Several types of dynorphin A exist, including dynorphin $A_{(1-17)}$ (Goldstein et al., 1981), dynorphin $A_{(1-8)}$ (Seizinger, Hollt and Herz, 1981), and other peptides of intermediate sizes (Suda, Tozawa, Tachibana, Demura and Shizume, 1982). Immunoreactive dynorphin perikarya are distributed in several cerebral cortical areas, striatum, amygdala, hippocampus, several hypothalamic nuclei (including supraoptic and paraventricular), midbrain periaqueductal gray, and numerous brainstem areas, such as the parabrachial and spinal trigeminal nuclei, NTS, lateral reticular nucleus, and in the dorsal horn of the spinal cord (Khachaturian et al., 1985). Comparison of the CNS distribution of the dynorphins and enkephalin immunoreactive cells and fibers show that these two systems often overlap.

II. Opioid Receptor Subtypes

Initial investigations of structure-activity relationships with literally thousands of synthetic and semi-synthetic opiate compounds (Jacobson, May and Sargent, 1970; Janssen, Hellerback, Schnider, Besendorf and Pellmont, 1960, 1966) provided strong early pharmacological evidence in favor of a receptor-mediated mechanism of opioid action. It was revealed that such a site exhibited strict stereospecificity (Portoghese, 1966, 1970), while in vivo testing and bioassays demonstrated cross-tolerance and dependence. Prior to 1973 however, attempts to label the receptor biochemically were unsuccessful. Three independent laboratories then demonstrated opioid binding

sites using radiolabeled naloxone (Pert and Snyder, 1973), dihydromorphine (DHM: Terenius, 1973), and etorphine (Simon, Hiller and Edelman, 1973). The first evidence for multiple opioid receptors was derived from the behavioral and neurophysiological observations in the chronic spinal dog preparation by Martin and his colleagues (Martin, Eades, Thompson, Huppler and Gilbert, 1976). They proposed the existence of three types to explain differential patterns of the opioid agonists, morphine, ketocyclazocine and SKF 10,047. Administration of morphine resulted in analgesia, miosis, bradycardia, hypothermia, and indifference to environmental stimuli. Following an injection of ketocyclazocine the dogs exhibited miosis, general sedation and a depression of flexor reflexes, while following SKF 10,047 mydriasis, increased respiration, tachycardia and delirium was evident. In addition to different classes of opiate drug-induced behavioral syndromes, tolerance to one group of opiates did not result in cross-tolerance to another class of opiate drugs. Based on these findings, three types of opioid receptors were proposed: mu for morphine-like compounds, kappa for ketocyclazocine-like drugs and sigma for drugs such as SKF 10,047. The sigma receptor was subsequently thought to be non-opiate since it was unaffected by the opiate antagonist, naloxone.

Further support for the idea of multiple opioid receptors came from studies using peripheral organ bioassays in which the relative potencies of several opioids and opiate antagonists varied as a function of the tissue system (Chang, Hazum and Cuatrecasas, 1981), again suggesting receptor heterogeneity. Lord and co-workers

(Lord et al., 1977) used a mouse vas deferens bioassay in which enkephalin peptides were found to be particularly potent, and suggested the existence of a delta opioid receptor. Observations of differential potencies and binding profiles of beta-endorphin, leu-enkephalin, and met-enkephalin on the guinea pig ileum and mouse van deferens bioassays (Lord et al., 1977) indicated the existence of three opioid receptor sites: mu, kappa, and delta. These findings were confirmed by homogenate binding and autoradiographic studies demonstrating that mu, delta and kappa receptors were distinct opioid binding sites (Goodman, Snyder, Kuhar and Young, 1980; Mansour, Lewis, Khachaturian, Akil and Watson, 1986). Opioid receptors are widely distributed throughout the neuraxis with particularly dense binding observed in limbic structures, thalamic nuclei and neural areas important for visceral functioning (Mansour, Khachaturian, Lewis, Akil and Watson, 1988).

In this and the following sections, agonists and antagonists for the different opioid receptors will be introduced. Naloxone and naltrexone are reversible and short-acting (1-4 h) opioid antagonists with greatest affinity for mu receptors, but also for delta and kappa receptors (see reviews: Sawynok, Pinsky and LaBella, 1979; Zukin and Zukin, 1981). Beta-chlornaltrexamine (B-CNA) is an irreversible opioid antagonist which acts by alkylating all opioid receptors (Portoghese, Larson, Sayre, Fries and Takemori, 1980).

1. Mu receptors. Mu opioid receptors are widely distributed throughout the forebrain, midbrain and hindbrain. They are most dense in the neocortex, caudate-

putamen, nucleus accumbens, thalamus, hippocampus, amygdala, inferior and superior colliculi, NTS, spinal trigeminal nucleus and the dorsal horn of the spinal cord. A moderate density of mu receptors is observed in the periaqueductal gray and raphe nuclei, while relatively little binding is seen in the hypothalamus, preoptic area and globus pallidus (Mansour et al., 1988).

There are both mu-selective agonists and antagonists. D-Ala^2,met-Phe^4, Gly(ol)^5-enkephalin (DAMGO) is a highly-selective mu agonist (Handa, Lane, Lord, Morgan, Rance and Smith, 1981). Beta-funaltrexamine (B-FNA) acts as a reversible agonist at the kappa receptor with subsequent irreversible antagonism at the mu receptor (Portoghese et al., 1980). Both the reversible kappa agonist actions and the irreversible mu antagonist actions of B-FNA have been confirmed pharmacologically and biochemically (Takemori, Larson and Portoghese, 1981; Ward, Portoghese and Takemori, 1982a,b, 1983).

a) Mu_1 and Mu_2 receptors. Two subtypes of mu receptors (mu_1 and mu_2) have been described (Wolozin and Pasternak, 1981). The mu_1 receptor has been characterized biochemically and pharmacologically using the mu_1-selective antagonists, naloxazone and naloxonazine (e.g., Pasternak and Wood, 1986; Wolozin & Pasternak, 1981; Hahn, Carroll-Buatti and Pasternak, 1982). The mu_1 receptor binds many opiates and enkephalins equally well while the mu_2 receptor subtype selectively binds morphine-like compounds more potently than enkephalins (Clark, Houghten and Pasternak, 1988; Pasternak and Wood, 1986). Naloxazone blocked

the high affinity binding of both [^{3}H]DHM and [^{3}H][D-Ala^2-D-Leu^5]-enkephalin (DADL) in saturation studies (Hahn et al., 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-ehtylmaleimide (Burkhardt, Frederickson and Pasternak, 1982; Nishimura, Recht and Pasternak, 1984; Pasternak, Gintzler, Houghten, Ling, Goodman, Spiegel, Nishimura, Johnson and Recht, 1983; Wolozin and Pasternak, 1981). There is also a good correspondence between the autoradiographic distribution of mu_1 receptors and supraspinal sites implicated in antinociception as revealed by pharmacological studies (Goodman and Pasternak, 1985; Moskowitz and Goodman, 1985). Autoradiographic studies demonstrate that mu_1 and mu_2 binding sites have similar, but not identical distributions (Moskowitz and Goodman, 1985; Goodman and Pasternak, 1985). Cortical mu_1 binding is denser in the frontal lobe, whereas mu_2 binding predominates in the parietal, occipital, and temporal cortices. Mu_1 binding is found in striatum, ventral pallidum, caudal nucleus accumbens, medial thalamus, interpeduncular nucleus and median raphe, whereas mu_2 binding is found in hippocampus and amygdaloid region. Additionally, mu_1 receptors are found in abundance in the ventral periaqueductal grey, while mu_2, but not mu_1, receptor binding is quite dense in the dorsal motor nucleus of the vagus nerve and the nucleus tractus solitarius. The differential distribution between mu_1 and mu_2 receptor subtypes provides anatomical correlates for the respective roles of these binding sites

in antinociceptive and respiratory depressant effects following opioid administration (Pasternak, Childers and Snyder, 1980).

The presence of distinct high-affinity binding sites suggests that opiate actions are mediated by the different receptor subtypes. Much evidence suggests that mu_1 sites mediate morphine antinociception (Pasternak and Wood, 1986) and play a role in supraspinal analgesia (Bodnar, Williams, Lee and Pasternak, 1988). Naloxazone and naloxonazine decrease morphine and other forms of opioid antinociception in rats and mice (Zhang and Pasternak, 1981; Ling and Pasternak, 1983). These effects were observed following DAMGO (Heyman, Mulvaney, Mosberg and Porecca, 1987) and the mu_1/delta agonist (D-Ser^2, Leu^5)-enkephalin-Thr^6 (DSLET) (Paul, Bodnar, Gistrak and Pasternak, 1989). Additionally, naloxonazine antagonized morphine and DSLET antinociception elicited from the PAG, locus coeruleus, nucleus raphe magnus, and the nucleus reticularis gigantocellularis (Bodnar et al., 1988). Also, mu_1 sites have been implicated in acetylcholine turnover, prolactin release, hypothermia, catalepsy, and some signs of physical dependence (Pasternak and Wood, 1986). In contrast to supraspinal antinociception, spinally-mediated antinociception seems to be mediated in part by the mu_2 receptor subtype. Naloxonazine pretreatment shifted the supraspinal DAMGO and DSLET antinociceptive dose-response curves to the right by 4-10 fold, but failed to alter their spinal antinociceptive effects (Heyman et al., 1987; Paul et al., 1989). However, intrathecal DAMGO antinociception was blocked by the irreversible mu antagonist,

B-FNA (Paul et al., 1989). Further, mu_2 sites are involved in respiratory depression, gastrointestinal transit, bradycardia, growth hormone release, and dopamine turnover. Thus, in studying the roles of mu_1 and mu_2 receptor subtypes in physiological function, the antagonists, B-FNA (mu: mu_1 + mu_2) and naloxonazine (mu_1) can serve as tools to differentiate receptor-mediated effects.

2. Delta receptors. Autoradiography indicates that delta opioid receptors appear densest in olfactory-related neural areas, neocortex, caudate-putamen, nucleus accumbens, and amygdala with little binding in the thalamus, hypothalamus and brainstem (Mansour et al., 1988). The opioid actions of delta receptors have been characterized in both spinal (e.g., Paul et al., 1989; Heyman et al., 1987) and supraspinal (Jensen and Yaksh, 1986; Porecca, Mosberg, Hurst, Hruby and Burks, 1984; Heyman et al., 1987) analgesic assays. The prototypical delta receptor agonists were met-enkephalin, leu-enkephalin and the latter's analogue, DADL (Lord et al., 1977). More specific delta receptor agonists were subsequently developed, including DSLET and D-Pen^2, D-Pen^5-enkephalin (DPDPE) (Mosberg, Hurst, Hruby, Gee, Yamamura, Galligan and Burks, 1983; Mosberg, Hurst, Hruby, Galligan, Burks, Gee and Yamamura, 1983) with the former, but not the latter, possessing mu_1 affinity (Itzhak and Pasternak, 1987; Clark et al., 1982). ICI 174864, an initial reversible and short-acting delta receptor antagonist (Cotton, Giles, Miller, Shaw and Timms, 1984), produces motor dysfunction (Long, Petras and Holaday, 1988). Naltrindole is a selective and reversible delta receptor antagonist (Portoghese, Sultana, Nagase

and Takemori, 1988). DALCE displays high affinity for and covalently binds to delta receptors, whereas it displays moderate and reversible affinity for mu receptors (Bowen, Hellewell, Kelemen, Huey and Steward, 1987). DALCE acts as a short-acting delta, and secondarily mu, agonist, and a long-lasting, selective delta antagonist in binding and pharmacological assays (Bowen et al., 1987; Calcagnetti, Bowen and Holtzman, 1989). Delta receptors have been putatively subdivided into $delta_1$ and $delta_2$ subtypes (Negri, Potenza, Corsi and Melchiori, 1991) with DALCE ($delta_1$: Jiang, Bowen, Mosberg, Rothman and Porreca, 1990) and naltrindole ($delta_2$: Portoghese, Sultana, Nagase and Takemori, 1988) respectively proposed as specific antagonists for each subtype.

3. Kappa receptors. Kappa opioid receptors have been most densely localized in the caudate-putamen, nucleus accumbens, amygdala, hypothalamus, neural lobe of the pituitary, median eminence, and NTS. Moderate levels of kappa opioid binding are found in the periaqueductal gray, raphe nuclei, spinal trigeminal nucleus and dorsal horn of the spinal cord (Mansour et al., 1988). Whereas U50,488H is the prototypical kappa agonist (VanVoigtlander, Lahti and Ludens, 1983), the most selective, though reversible antagonist is nor-binaltorphamine (Nor-BNI: Portoghese, Lipkowski and Takemori, 1987). Nor-BNI's antagonist effects upon kappa receptors have been confirmed in that it has a 170-fold greater affinity for kappa, relative to mu, receptors (Takemori, Ho, Naeseth and Portoghese, 1988). The kappa receptor has been recently subdivided into K_1, K_2 and K_3 binding sites (e.g., Zukin, Eghbali,

Olive, Unterwald and Tempel, 1988; Rothman, Bykov, deCosta, Jacoboson, Rice and Brady, 1990; Clark, Liu, Price, Hersh, Edelson and Pasternak, 1989). U50,488H and Nor-BNI respectively produce their most potent agonist and antagonist activities at the K_1 site. The K_3 site has been identified in both binding and pharmacological studies using naloxone benzolhydrazone (NalBzoH), a selective K_3 agonist and weak mu antagonist (Clark et al., 1989; Gistrak, Paul, Hahn and Pasternak, 1989; Paul, Levison, Howard, Pack, Hahn and Pasternak, 1990).

III. Opioid receptor interactions with opioid ligands

1. Endogenous opioid ligands. Early investigators examining the complexity of distributions of both the opioid receptors and opioid peptide families in the CNS made an assumption that, based on the relative selectivities of the opioid peptides, there should be a correspondence between a particular opioid peptide and receptor subtype. Enkephalins and dynorphins bind preferentially to delta and kappa opioid receptors respectively in in vitro experiments. Beta-endorphin binds to mu and delta, but not kappa, opioid receptors (Robson, Paterson and Kosterlitz, 1980). However, these in vitro associations did not correlate well with existing anatomical relationships. Proenkephalin and prodynorphin peptides can bind to mu, delta or kappa receptors depending on the specific peptide product and on the species (Corbett, Paterson, McKnight, Magnan and Kosterlitz, 1982). Mu and kappa opioid receptors are extensively co-localized in the rat brain and are distributed similarly to enkephalin immunoreactivity in many areas, including cortex, habenula,

interpeduncular nuclear complex, parabrachial nuclei, NTS, spinal trigeminal nucleus, and dorsal horn of the spinal cord (Quirion, Bowen, Herkenham and Pert, 1982). Such findings indicated that the ligand-receptor anatomical associations predicted by in vitro studies appear incorrect. Instead, since it is now known that some extended proenkephalin peptides have substantial kappa and mu properties (Quirion et al., 1983), while dynorphin A loses kappa selectivity upon C-terminal cleavage, the apparent availability of multiple opioid receptors to each opioid system may signify that differential processing of the opioid precursor is a biological strategy for yielding peptide products which act at the different receptors (Mansour et al., 1988).

2. Exogenous opioid ligands. Because of the difficulty in demonstrating the role of opioid system using endogenous ligands, the search for more selective opiate agonists and antagonists, as well as tissue preparations containing purer receptor types was necessary. The use of those selective ligands aided in understanding of specific receptors pharmacological characteristics. In the rat the following receptor interactions have been suggested:

mu: morphine = beta-endorphin = enkephalins > dynorphin

mu_1: morphine = enkephalin = DAMGO = DSLET = DADL = beta-endorphin = EKC > > Dynorphin > > DPDPE

delta: DPDPE > enkephalins = DSLET = DADL > morphine > dynorphin

kappa: dynorphin > EKC > > morphine > enkephalin

Studies into the function of endogenous opioid peptide families and multiple

opioid receptor subtypes have included their evaluation in antinociceptive, euphoric, addictive, respiratory, gastrointestinal and reward processes. Since the aim of this dissertation is the evaluation of opioid receptor subtype roles in ingestion of palatable fluids, the following section will limit itself to the roles of general and specific opioid receptor agonists and antagonists upon ingestive behavior.

IV. Opioids and feeding behavior

Initial observations (Flowers, Dunham and Barbour, 1929; Barbour, Gregg and Hunter, 1930) that opiates influence ingestive behaviors revealed that food and water intake increased in rats following morphine withdrawal, and that morphine increased basal metabolic rate in dogs, suggesting opioid involvement in both energy expenditure and energy intake. Martin and colleagues (Martin, Wikler, Eades and Pescor, 1963) subsequently found that morphine-addicted rats initially decreased food intake, but then increased intake following daily morphine injections. Initial roles for endogenous opioids in feeding were also confirmed by increased intake following beta-endorphin injections into the ventromedial hypothalamus (Grandison and Guidotti, 1977).

1. Endogenous opioid ligands and feeding. Since the report of Grandison and Guidotti (1977) indicating that beta-endorphin stimulates feeding, further studies examined which ligands mediated opioid-induced feeding. Leibowitz and Hor (1982) found that beta-endorphin stimulated intake (1 g/h) when infused into the lateral ventricle and paraventricular or ventromedial hypothalamus. This hyperphagia was

antagonized by the alpha-adrenergic receptor antagonist, phentolamine, which suggested that this opioid effect might be the result of stimulation of the noradrenergic feeding system. The proenkephalin derived products met- and leu-enkephalin also induced feeding after injection into the ventromedial hypothalamus (Tepperman and Hirst, 1983). The long-acting structural enkephalin analogs, DADL and D-Ala-met-enkephalinamide, also stimulate feeding (1.5-2.5 g/h) (McLean and Hoebel, 1983).

Following the initial observation that dynorphin increased feeding in mice (Walker, Katz and Akil, 1980), it was found that dynorphin increased feeding in non-deprived rats during the light cycle (1.5 g/h) (Morley and Levine, 1981). The hyperphagic effects of dynorphin appear specific since dynorphin fails to alter drinking, resting or locomotor behaviors (Levine et al., 1985). While dynorphin $A_{(1-17)}$ appeared more effective in stimulating feeding than dynorphin $A_{(1-13)}$, naloxone decreased both of their ingestive effects (Morley and Levine, 1983). Another approach in evaluating opioid effects upon feeding involved intracerebral administration of antibodies raised against specific opioid peptides (Schultz, Wilhelm and Dirlich, 1984). Administration of an alpha-neo-endorphin antibody into the ventromedial hypothalamus inhibited both food and water intake. Dynorphin antibodies produced similar, but less pronounced, effects, while beta-endorphin antibodies significantly reduced feeding and drinking following administration into the periventricular nucleus of the hypothalamus. In contrast, feeding induced by

electrical stimulation of the lateral hypothalamus was inhibited by administration of antibodies raised against dynorphin $A_{(1-13)}$, but not beta-endorphin (Carr, Bak, Gioannini and Simon, 1987).

2. General opioid antagonists and feeding. The involvement of endogenous opioid peptides in feeding was also examined in general antagonist studies. Holtzman (1974) first demonstrated that naloxone decreased food and water intake in rats. Subsequent studies have found that naloxone decreases feeding in rats and mice under spontaneous (Jalowiec, Panksepp, Zolovick, Najam and Herman, 1981) and nocturnal (Brands, Thornhill, Hirst and Gowdey, 1979; Jalowiec et al., 1981; Lowy, Maickel and Yim, 1980) conditions and following food deprivation (Brands et al., 1979), norepinephrine (Morley, Levine, Murray and Kneip, 1982), muscimol (Morley, Levine and Kneip, 1981), 2-deoxy-D-glucose (Lowy et al., 1980), insulin (Lowy et al., 1980; Levine and Morley, 1981; Ostrowski, Rowland, Foley, Nelson and Reid, 1981; Rowland and Bartness, 1982), stress (mild tail-pinch and social conflict) (Lowy et al., 1980; Morley and Levine, 1980), and hypothalamic stimulation-induced feeding (Jenck, Gratton and Wise, 1986). Naloxone has been shown to reduce both the size and duration of the first meal and increase the first post-meal interval (McLaughlin and Baile, 1984). In studies of macronutrient self-selection, naloxone selectively reduces fat consumption, while having little effects upon other macronutrients (Marks-Kaufman and Kanarek, 1981, 1990; Marks-Kaufman, Plager and Kanarek, 1985). Further, whereas food deprivation increases

hypothalamic beta-endorphin levels (Majeed, Lason, Przewlocki and Przewlocki, 1986), tail-pinch feeding stress decreases immunoreactive dynorphin levels in cortex, but not hypothalamus (Morley, Elson, Levine and Shafter, 1982).

A number of investigators have examined the effects of chronic administration of opioids and observed their effects on food intake and body weight. Chronic administration of naloxone abolished dietary-induced obesity in rats fed a high-fat cafeteria type diet and increased their metabolic rate (Margules, Moisset, Lewis, Shibuya and Pert, 1978). In contrast, animals consuming a low-fat control diet failed to respond to this chronic naloxone. The authors suggested that this effect may be due to blockade of the opioid stimulation of appetite by palatable food and also by reducing energy expenditures. Another study (Marks-Kaufman, Balmagiya and Gross, 1984) reported that chronic naltrexone infusions (200 ug/kg/hr for 14 days) decreased appetite and reduced energy production in rats fed chow plus a 32% sucrose solution. Similar results of a lesser magnitude were seen in the animals fed chow only. In addition, genetically obese (ob/ob) mice treated with naltrexone (10 mg/kg) twice a day from post-natal weeks 7 to 12, lost weight (Recant, Voyles, Lucian and Pert, 1980) while no effect of naltrexone was noted in the lean littermates. Using a slow release naltrexone capsule, Lang and colleagues (Lang, Strehlendorf, Strahlendorf and Barnes, 1981) found no effect of naltrexone on food intake, water intake, urine output, fecal output, and body weight over a thirty day period in lean rats. Chronic administration of naloxone also decreased food intake

and body weight in adult rats, decreased food intake and body weight gain in adolescent rats, but failed to alter intake or weight in dietary-obese rats maintained on a cafeteria diet (Mann, Pasternak, Hahn, Curreri, Lubin and Bodnar, 1988). B-CNA, which irreversibly alkylates all opioid receptors reduces food intake and body weight as well (Gosnell, Grace and Levine, 1987). Finally, a series of trans-3,4-dimethyl-4-phenylpiperidine compounds which act as long-term general opioid antagonists (e.g., LY255582: Leander, Hart, Lochner, Hynes and Zimmerman, 1982) reduce food intake and weight gain in normal (Shaw, Mitch, Leander and Zimmerman, 1990) and genetically-obese rats (Levine, Grace and Billington, 1991).

In human studies, naloxone decreased intake during a single meal in both lean and obese subjects (Atkinson, 1982), and also decreased feeding induced by 2DG (Thompson, Welle, Lilivavat, Penicaud and Campbell, 1983). On the other hand, chronic administration of naltrexone to obese humans failed to alter body weight (Levine et al., 1985).

3. Opioid receptor subtype agonists and feeding. With the development of specific opioid agonists that bind selectively to particular receptor subtypes, it was possible to examine the role of those receptors in feeding. Since dynorphin has been considered to be the prototypical ligand for the kappa receptor (Chavkin, James and Goldstein, 1982) and since dynorphin stimulated feeding, it was proposed that the kappa receptor is integral in the modulation of opioid-induced feeding (Cooper, Jackson and Kirkham, 1985; Morley, Levine, Kneip, Grace, Zeugner and Shearman,

1985). Additional studies investigating the role of the kappa receptors have been conducted using exogenous opiates which preferentially bind to the kappa opioid receptors. The cyclazocine and ketocyclazocine compounds, stimulated feeding during the light phase of the cycle in non-deprived rats (Morley, Levine, Grace and Kneip, 1982). More selective kappa agonists, including bremazocine, butorphenol and U50,488H also enhance feeding in rats (Gosnell, Levine and Morley, 1986; Gosnell, Morley and Levine, 1986; Levine and Morley, 1983; Morley and Levine, 1983). Gosnell and colleagues compared the central hyperphagic actions of kappa-selective (dynorphin $A_{(1-17)}$), mu-selective (DAMGO), and delta-selective (DSLET) opioid agonists, and found that the rank-order potency for stimulation of food intake in non-deprived rats was dynorphin > > DAMGO > DSLET (Gosnell et al., 1986).

Roles for the mu receptor in opioid-induced feeding were initially evaluated with morphine. Whereas morphine stimulated feeding in non-deprived rats, it actually decreased feeding following food deprivation (Sanger and McCarthy, 1980). Indeed, repeated daily administration of morphine produces a more reliable feeding response, an effect attributable to the development of tolerance to morphine's depressant effects (Levine, Murray, Kneip, Grace and Morley, 1982; Woods and Leibowitz, 1985). Whereas one specific mu agonist, morphiceptin, failed to induce feeding (Morley, Levine, Gosnell and Billington, 1984), the mu-selective agonist, DAMGO does elicit feeding responses (Gosnell et al., 1986). Mu-mediated and kappa-mediated feeding responses appear to act independently of one another based

upon cross-tolerance studies (Levine et al., 1985). Chronic administration of bremazocine over nine days elicited significantly more food intake than acute bremazocine treatment. In contrast, when animals received bremazocine over eight days and were administered morphine on the ninth day, the enhancement in feeding failed to occur. Similar results occurred when morphine was injected over eight days and bremazocine was administered on the ninth day.

Several studies have reported that the administration of morphine causes a selective increase in the intake of dietary fat (e.g.: Marks-Kaufman, 1982; Marks-Kaufman and Kanarek, 1980; Marks-Kaufman and Lipeles, 1982). Another opioid agonist, butorphanol, has also been found to cause preferential increases in the intake of a high-fat diet (Rosmos, Gosnell, Morley and Levine, 1987), suggesting involvement of mu opioid receptor in modulation of fat intake. More recently, Evans and Vaccarino (1990) reported that morphine increased the intake of protein-rich diet in protein-deprived rats, and slightly increased intake of a carbohydrate-rich diet in carbohydrate-deprived rats. Marks-Kaufman and Kanarek (1990) have pointed out that morphine selectively increases fat intake even when fat is not the most preferred nutrient (Marks-Kaufman, 1982). In contrast, Gosnell and coworkers (Gosnell, Krahn and Majchrzak, 1990) reported that large individual differences in diet selection allowed the identification of groups of rats characterized as either fat-preferring or carbohydrate-preferring, and this preference was found to correlate with the changes in food intake and diet selection observed after systemic injections of

morphine. The effect of morphine on diet selection appears to be influenced by the deprivation status of the animals. The selective increases in fat intake reported by Marks-Kaufman (1982) and Marks-Kaufman and Kanarek (1990) were obtained with rats on a restricted feeding schedule. Shor-Posner and coworkers (Shor-Posner, Azar, Filart, Tempel and Leibowitz, 1986) obtained similar results in food-restricted rats, but found that in non-deprived rats, morphine selectively increased protein intake. Bhakthavatsalam and Leibowitz (1986) reported that in non-deprived rats, morphine increased the intake of all three macronutrients, although the increases in fat and protein intake were greater than that of carbohydrate intake. On the other hand, two studies with chronic morphine exposure reported exclusive increases in fat intake in non-deprived rats (Marks-Kaufman and Lipeles, 1982; Ottaviani and Riley, 1984).

Since considerable overlap exists between particular opioid agonists and different receptor subtypes, and since higher agonist doses are needed to achieve pharmacological as compared to biochemical effects, it is somewhat difficult to ascertain precise roles of different opioid receptor subtypes in different types of feeding behavior based on agonist studies. It is also difficult to ascertain subtype mediation of intake with naloxone and naltrexone since they are short-acting, and fail to clearly distinguish among mu, delta and kappa subtypes (e.g., Zukin and Zukin, 1981; Pasternak and Wood, 1986).

4. Opioid receptor subtype antagonists and feeding. One of the major advantages of selective opioid antagonists over selective agonists is their utility in

probing the interaction of endogenous opioid peptides and new opioid agonists with opioid receptor types. Moreover, since it is sometimes not easy to distinguish among mu, delta, and kappa opioid receptor-mediated agonist effects if the pharmacological endpoints are identical (e.g. antinociception or inhibition of a smooth muscle preparation by agonists), selective antagonists have wider utility as tools than selective agonists (Portoghese, 1991). With the development of specific opioid antagonists that bind selectively to particular receptors it was possible to examine the role of those receptors in feeding. The following sections evaluate specific opioid receptor subtype antagonist effects upon: a) free feeding, b) opioid agonist-mediated hyperphagia, c) deprivation-induced feeding, d) glucoprivic feeding, e) tail-pinch feeding, f) lesion-induced feeding and g) high-fat feeding.

a) Free feeding. B-FNA, a short-acting kappa agonist, and irreversible mu antagonist (Takemori et al., 1981) respectively stimulated free feeding for 2 h and inhibited free feeding and body weight after 24 h (Ukai and Holtzman, 1988). Arjune and co-workers (Arjune et al., 1990) also observed that B-FNA significantly increased food intake for 6 h, reduced food intake (35-41%) and body weight (5-7%) after 24-72 h, and decreased mu binding in striatum (89%) and hypothalamus (46%). It was also found (Simone, Bodnar, Goldman and Pasternak, 1985) that the mu_1 antagonist, naloxonazine significantly decreased free feeding (32%) after 24 h, whereas chronic naloxonazine treatment significantly reduced intake (21%) and weight (7%) in adult rats and intake (24%) and weight gain (53%) in adolescent rats

(Mann, Arjune, Romero, Pasternak, Hahn and Bodnar, 1988). Nor-BNI, a reversible kappa antagonist (Takemori et al., 1988) significantly suppressed spontaneous nocturnal intake at the onset (2 h) of the dark cycle (54%; Arjune and Bodnar, 1990). The reversible delta receptor antagonist, ICI 174864 transiently decreased nocturnal intake only at doses (10-30 ug: 14-62%; Jackson and Sewell, 1985) that produce motor dysfunction (Long et al., 1988; Islam and Bodnar, 1990). The short-acting delta agonist actions of DALCE (Bowen et al., 1987) significantly increased free feeding for up to 10 h, but its long-acting delta antagonist actions failed to significantly alter intake (20% decrease) or weight (3.5% decrease) after 24-72 h (Arjune, Bowen and Bodnar, 1991). These data suggest opioid antagonist efficacies upon free feeding of: general = kappa > mu = mu_1 >> delta.

b. Opioid agonist-mediated hyperphagia. Hyperphagia following mu, delta and kappa agonists are blocked by naloxone, naltrexone or B-CNA (Gosnell et al., 1987; Morley and Levine, 1981; Sanger and McCarthy, 1981; Stanley, Lanthier and Leibowitz, 1989). The kappa antagonist, Nor-BNI blocked opioid-mediated hyperphagia induced by kappa-selective (U50,488H), mu-selective (DAMGO) and delta-selective (DSLET) agonists as well as hyperphagia induced by hypothalamic electrical stimulation (Levine, Grace, Billington and Portoghese, 1990; Carr, Bak, Simon and Portoghese, 1989). Further, the short-acting hyperphagic responses induced by B-FNA and DALCE were blocked by Nor-BNI, but not by B-FNA (Arjune et al., 1990, 1991). Thus, it appears that kappa, delta and mu agonists

depend upon an active kappa site for expression of their hyperphagic effects (Levine et al., 1990). In contrast, delta, mu and mu_1 antagonists blocked only those hyperphagic responses induced by their respective agonists. ICI174864 reduced DADL hyperphagia (Jackson and Cooper, 1985). B-FNA significantly reduced DAMGO hyperphagia, but not DSLET or U50,488H hyperphagia (Levine et al., 1991). Naloxonazine blocked morphine hyperphagia, but not ethylketocyclazocine, dynorphin or DADL hyperphagia (Mann et al., 1988).

c. Deprivation-induced feeding. Hyperphagia following food deprivation is decreased by central administration of mu, mu_1 and kappa receptor antagonists. Deprivation-induced feeding was significantly reduced by B-FNA (33-49%) and naloxonazine (34%) (Arjune et al., 1990; Simone et al., 1985; Levine et al., 1991), but was only marginally reduced by Nor-BNI (kappa, 28%), DALCE ($delta_1$, 18-28%) and naltrindole ($delta_2$) (Arjune et al., 1991; Levine et al., 1990; AS Levine, personal communication). These data suggest opioid antagonist efficacies upon deprivation-induced feeding of: general = mu > mu_1 > kappa > > delta.

d) Glucoprivic feeding. 2DG and insulin hyperphagia have been dissociated in terms of both central mechanisms (see: Beczkowska and Bodnar, 1991) and opioid receptor subtype antagonist effects. 2DG hyperphagia is reduced following central administration of B-FNA (100%) and Nor-BNI (68%) in a manner similar to that of central naltrexone (69%; Arjune and Bodnar, 1990; Arjune et al., 1990). In contrast, 2DG hyperphagia was unaffected by the delta antagonists, ICI 174864 (13% decrease:

Jackson and Sewell, 1985) and DALCE (19-31% increase: Arjune et al., 1991), and increased by the mu_1 antagonist, naloxonazine (23-39%: Simone et al., 1985). The effectiveness of B-FNA (mu_1 + mu_2) and the inetfectiveness of naloxonazine (mu_1) suggest a role for the low-affinity mu_2 receptor in 2DG hyperphagia. These data suggest opioid antagonist efficacies upon 2DG hyperphagia of: mu_2 > general = kappa > > delta > mu_1.

In contrast, it was found (Beczkowska and Bodnar, 1991) that insulin hyperphagia over 6 h was significantly reduced by B-FNA (54%), but only marginally reduced by naltrexone (30%), Nor-BNI (27%), DALCE (8%) or naloxonazine (30%). These data suggest opioid antagonist efficacies upon insulin hyperphagia of: mu_2 > > general = kappa = mu_1 > delta.

e) Tail-Pinch feeding. Microinjections into the substantia nigra of either naltrexone or Cys^2-Tyr^3-Orn^5-Pen^7-amide (CTOP) significantly reduce tail-pinch feeding without reducing gnawing; naltrindole and Nor-BNI failed to exert effects (Hawkins, Cubic, Baumesiter and Bartin, 1992). To determine whether mu_1 or mu_2 receptors and $delta_1$ or $delta_2$ receptors were involved, Koch and Bodnar (1992) found that tail-pinch feeding was significantly reduced by central pretreatment with B-FNA (29%) and naloxonazine (32%), but was unaffected by either Nor-BNI (15%), DALCE (19%) or naltrindole (30% increase). The reductions in tail-pinch food intake were not accompanied by changes in the duration of intake, indicating that the mu antagonists were modulating ingestive rather than activational mechanisms.

f) Lesion-Induced Hyperphagia. Opioid microinjections into the rat hypothalamic PVN produce agonist stimulation and antagonist inhibition of food intake (Gosnell et al., 1986; Stanley et al., 1989; Woods and Leibowitz, 1985). Lesions placed in the hypothalamic PVN increase body weight and food intake (e.g., Leibowitz, Hammer and Chang, 1981; Shor-Posner et al., 1986). Evaluation of opiate receptor antagonists upon food intake in rats with hyperphagia-producing lesions has been limited to the failure to alter naloxone hypophagia in rats with lesions placed in the ventro-medial hypothalamic nucleus (King, Castgellanos, Kastin, Berzas, Mauk, Olson and Olson, 1979). It was recently observed that rats with lesions placed in the hypothalamic PVN significantly increased food intake and body weight for up to seven weeks after the lesion, and displayed significant reductions in the magnitude and potency of central naltrexone (10-50 ug, icv) hypophagia (R. Bodnar, unpublished observations).

g) High-Fat feeding. Hyperphagia following access (1-2 h) to palatable foods is stimulated by kappa agonists (e.g., Morley and Levine, 1981; Morley and Levine, 1983; Cooper et al., 1985; Morley et al., 1985), and is selectively reduced by opioid receptor subtype antagonists. High-fat intake is significantly reduced by systemic (67%) and central (51%) naloxone and naltrexone as well as by central Nor-BNI (79%) and B-FNA (37%) (Islam and Bodnar, 1990; Arjune and Bodnar, 1990). The failure of naloxonazine (9% increase) to alter high-fat intake (Islam and Bodnar, 1990) indicates a role for the mu_2 receptor subtype. Whereas the reductions (41%)

in high-fat intake by ICI 174864 were accompanied by motor dysfunction (Islam and Bodnar, 1990), DALCE failed to reduce high-fat intake (2-24%; Arjune et al., 1991). These data suggest opioid antagonist efficacy upon high-fat intake of: kappa > general > mu_2 > delta > mu_1.

V. Rationale

Like exposure to a high-fat diet, intake is increased following exposure to palatable sweet solutions (e.g., sucrose or saccharin). Whereas naloxone reduces both sucrose and saccharin intake, mu and delta opioid agonists stimulate these forms of intake (e.g., Cooper, 1983; Lynch and Libby, 1983; Siviy and Reid, 1983; Gosnell and Majchrzak, 1989). Indeed, naloxone shifted the concentration-dependent increases in sucrose intake to the right in sham-feeding animals (Kirkham and Cooper, 1988a,b; Rockwood and Reid, 1982). Opiate agonists are more effective at increasing the intake of palatable foods than less palatable foods (Shor-Posner et al., 1986). The opiate antagonist naloxone is more effective at reducing intake of sweetened solutions (0.2% saccharin or 2.0% sucrose) than unadulterated tap water (Levine et al., 1982). Thus, the opiates may have a role in the control of palatable food consumption (Jackson and Cooper, 1985) which might be associated with modulating the hedonic value of food (Levine et al., 1985).

With the exception of high-fat diet studies employing selective antagonists for specific opioid receptor subtypes, there is no conclusive data elucidating which of the opioid receptor subtypes are involved in the modulation of palatable food intake.

The aim of this dissertation is to evaluate the role of specific endogenous opioid receptor subtypes (mu_1, mu_2, kappa, $delta_1$, and $delta_2$), by using their selective antagonists (naltrexone, B-FNA, Nor-BNI, naloxonazine, DALCE and naltrindole) in their respective modulation of intake of palatable liquid ingestates. The following palatable liquid ingestates were selected: sucrose (10%), saccharin (0.1%), and carbohydrate maltose dextrin (CMD; 10%).

Sucrose is a prototypical palatable liquid used in testing the reward value of food. Non-deprived animals representing a very wide variety of ecological niches consume sugar solutions readily and show preferences for them. Most plants contain enough sugar to be detectable by many terrestrial mammals. Indeed, sugars are the most abundant class of solutes in plants (Ramirez, 1990). The addition of a concentrated sucrose solution as a supplement to a chow diet increases weight gain in rats since they typically consume a large proportion of their calories as sucrose and increase caloric intake (Ackroff and Sclafani, 1988). The reasons for such increases in intake have been attributed to the detectable hedonic qualities of sugars rather than to any nutritional value (Ramirez, 1990) as sweetness is not correlated with the energy value of different sugars.

Endogenous opioid function was initially implicated in modulating intake of sweet solutions based upon the ability of the general opioid receptor antagonists, naloxone and naltrexone, to reduce intake of sweet solutions (Cooper, 1983; LeMagnen, Marfaing-Jallat, Micelli and Devos, 1980; Lynch, 1986; Lynch and

Libby, 1983; Sclafani, Aravich and Xenakis, 1982; Siviy and Reid, 1983). A further line of experiments has shown that opiate receptor antagonists may attenuate the reward of palatable fluids, and thereby reduce the preference for these solutions in two-bottle tests (LeMagnen et al., 1980). Comparable data have been seen in studies which have used non-deprived, food-deprived, or water-deprived animals. An animal's preference for the consumption of sweet solutions compared to water was consistently blocked by opiate antagonist treatments. The antagonists may have acted, therefore, to reduce the reward which normally derives from the palatable, sweet taste (Cooper, Jackson, Morgan and Carter, 1985).

Rats with gastric fistulas also display significant reductions in intake of sweet solutions following naloxone (Kirkham and Cooper, 1988a,b; Rockwood and Reid, 1982). The antidipsogenic effects of naloxone appear to be more potent in inhibiting sweet fluid intake than water intake (Levine et al., 1982). Kirkham and Cooper (1988a,b) suggest that naloxone's inhibition of sucrose intake is most probably due to its attenuation of palatability and not postingestive factors. These results however do not exclude the possibility that there could be a differential involvement of specific opioid receptor subtypes in modulation of fluid intakes that have palatable and nutritive characteristics as compared with those that are palatable but non-nutritive.

To separate nutritional factors from palatability factors, another experiment evaluated saccharin which has a sweet taste but is non-nutritive. This artificial

sweetener has been reported to taste bitter in humans and rats (Morrison and Jessup, 1977; Collier, 1962; Collier, 1967). However, Stewart and Krafczek (1988) reported that rats did not generalize conditioned saccharin aversion to bitter stimuli suggesting that saccharin, at low concentrations, might not taste bitter to rats.

Opioid involvement in the modulation of saccharin intake was demonstrated by naloxone's (1 mg/kg) marked attenuation of the consumption of a 0.05% saccharin solution in a two-bottle preference test. Water intake was simultaneously elevated, so that the naloxone-treated animals no longer expressed the preference for the sweet fluid (LeMagnen et al., 1980). Further, CXBK mice which are deficient in brain opioid receptors, consume less saccharin than controls (Yirmaya, Lieblich and Liebeskind, 1988). In contrast, intake of sweet solutions is increased following administration of morphine, the mu-selective opioid agonist, DAMGO, and the delta-selective opioid agonist [D-Thr2]-enkephalin-Thr6 (DTLET) (Calcagnetti and Reid, 1983; Czirr and Reid, 1986; Gosnell and Majchrzak, 1989; Lynch and Libby, 1983; Siviy, Calcagnetti and Reid, 1982).

There is now considerable evidence that rats as well as other animals have more than one type of sweet taste receptors (Beidler and Tonosaki, 1984; Jakinovich and Sugarman, 1988). As many as six different sugar receptors and a saccharin receptor exist in mammals based upon single-unit recording studies, taste blockers, taste mixtures, and cross-adaptation studies (Jakinovich and Sugarman, 1988). However, the degree to which activation of these different receptors produces similar

sweet taste sensations is not certain. Furthermore, some substances, in addition to stimulating sweet receptors, stimulate other taste receptors and thus produce complex taste sensations. There are four types of carbohydrates: 1) simple sugars (monosaccharides), such as fructose and glucose, 2) sugars, such as sucrose which consists of glucose and fructose, 3) polysaccharides, such as CMD which are more complex than sugars and are soluble in water, and 4) starches, which are complex and not soluble in water. Starch-derived polysaccharides are relatively tasteless to humans and have long been assumed to be bland-tasting to animals as well. Recent studies, however, demonstrate that rats, and probably other species, have a well-developed taste for starch-derived polysaccharides (Sclafani, 1987). Additional findings indicate that the taste sensation evoked by polysaccharides differs from that produced by sucrose. That is, rats conditioned to avoid Polycose solutions (a polysaccharide) display little or no avoidance of sucrose solutions (Nissenbaum and Sclafani, 1987). Consistent with these behavioral findings, electrophysiological recordings from the NTS indicate that the pattern of neural activity evoked by Polycose is quite distinct from that evoked by sucrose (Giza, Antonucci, Sclafani, and Scott, 1988).

The discovery of the rat's polysaccharide taste sensitivity emerged from experiments conducted with Polycose, a corn starch hydrolysate. Rats readily consume Polycose solutions and, at low concentrations, prefer Polycose to sucrose, glucose, fructose, and maltose solutions (Sclafani and Clyne, 1987; Sclafani and

Mann, 1987). Furthermore, the preference threshold for Polycose is 26 times lower than that for sucrose and maltose (Ramirez, 1991; Sclafani and Nissenbaum, 1987). Preference tests conducted with glucose polymers of different sizes further indicate that rats prefer polymers containing 4 to 8 glucose units relative to either smaller polymers or larger polysaccharides (Sclafani, Hertwig, Vigorito, Sloan and Kerzner, 1987). These findings indicate that it is the taste of the polysaccharides and not the small amounts of simple sugar in Polycose that rats find attractive and that rats have "polysaccharide" taste receptors which have several glucose subsites (Sclafani, 1987). Based on this evidence, Sclafani (1987) proposed that rats have two different taste systems for carbohydrates: a sweet taste system for the detection of sugar-rich foods, and the polysaccharide taste system which presumably is not stimulated by intact water-soluble starch molecules. This latter system is activated by smaller, soluble polysaccharide molecules cleaved from starch by the action of salivary amylase. Sclafani (1991) further proposed that the brain analyzes the incoming gustatory information in terms of taste quality and intensity. A sugar or polysaccharide taste sensation evokes an immediate and unconditioned hedonic response. If the carbohydrate source is very dilute (or if it is a sugar substitute, i.e., saccharin) the sweet or polysaccharide taste will soon lose its hedonic appeal and the animal will stop eating. If the carbohydrate source is not dilute, its taste will drive the animal to consume sufficient amounts to activate postingestive satiety mechanisms. Gastrointestinal and postabsorptive detectors appear to respond to the carbohydrate

load and send satiety signals to the brain. These signals then reduce the hedonic evaluation of the carbohydrate taste, in part by reducing the perceived taste intensity, to the point that the taste is no longer able to sustain ingestive behavior.

These data suggest that polysaccharides are equally, if not more, palatable to a rat than sugars. Since the opioid system, based on agonist and nonspecific antagonist studies, has been shown to be involved in the modulation of intake of sugar and saccharin solutions presumably by affecting their hedonic properties, it is reasonable to expect that an opioid system is involved in the modulation of intake of a palatable polysaccharide solution. To test this hypothesis, the polysaccharide carbohydrate maltose dextrin will be tested to answer the following questions: 1) Is the involvement of the opioid system in the modulation of palatable intake specific to sugars or does it extend to polysaccharides? 2) Is the involvement of the opioid system specific to a sweet taste or does it extend to other taste sensations?

It is important to determine whether opioid effects upon intake of sweet solutions employed in this dissertation are due to either orosensory alterations in the perceived palatable-hedonic qualities of the solutions, or alterations in the amount of fluid intake. Naloxone and naltrexone suppress fluid intake under both spontaneous and water deprivation conditions (e.g., Brown and Holtzman, 1979; Holtzman, 1975, 1979; Maickel, Braude and Zabik, 1977; Millan and Morris, 1988). Naloxone's effects are stereospecific (Brown and Holtzman, 1981; Cooper and Turkish, 1983), and are centrally-mediated (Brown and Holtzman, 1981; Czech, Stein

and Blake, 1983; Siviy, Bermudez-Rattoni, Rockwood, Dargie and Reid, 1981; Ukai and Holtzman, 1987). Opioid agonist effects appear more complex. Water intakes under spontaneous and deprivation conditions are decreased by morphine (Chance and Rosecrans, 1977; Cooper, 1980, 1981; Czech, Blake and Stein, 1984; Sanger and McCarthy, 1980) and enkephalin analogues (DeCarro, Micossi and Venturi, 1979; Spencer, Deupree, Hsiao, Mosberg, Hruby, Burks and Porreca, 1986; Summy-Long, Rosella and Keil, 1981). In contrast, kappa-selective agonists increase water intake under spontaneous conditions (Cooper et al., 1985; Sanger and MaCarthy, 1981; Turkish and Cooper, 1984). Ukai and Holtzman (1988a) found that intrahypothalamic administration of the mu-selective opioid agonist, DAMGO, increased spontaneous water intake, but decreased intake following water deprivation. Neither the kappa-selective agonist, dynorphin $A_{(1-13)}$ nor the delta-selective agonist, DPDPE, altered water intake. Since sucrose, saccharin and CMD will be presented in a liquid form, it is important to determine whether any selective opioid antagonist effects were due to a decrease in fluid intake. To this end, the effects of general and specific opioid receptor subtype antagonists upon water intake in water-deprived rats were examined.

CHAPTER 2: GENERAL METHODS

1. Subjects. Adult male albino Sprague-Dawley rats were purchased from Charles River Laboratories, Wilmington, MA, (80-120 days of age) and were housed individually in wire mash cages and maintained on a 12 h light - 12 h dark cycle with Purina rat chow and water available ad libitum. The rats that did preserve their cannulae throughout the study underwent all doses of the given antagonist and vehicle. The rats who lost their cannulae or whose cannulae lost their patency were replaced with new subjects in order to bring the number or rats in a given drug condition to the suitable n. It must be emphasized that all rats employed in this study were tested under vehicle conditions and that the animals did not differ from each other for a given condition.

2. Surgery. Each rat was pretreated with chlorpromazine (3 mg/kg, i.p.) and anesthetized with ketamine-HCL (100 mg/kg, i.m.). A stainless steel guide cannula (22-gauge, Plastic Products) was placed stereotaxically (Kopf Instruments) 0.3 mm above the left ventricle by using the following coordinates: incisor bar at (+) 5 mm, 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture and 3.6 mm from the top of the skull. The cannula was secured to the skull by 3 anchor screws with dental acrylic. All animals were allowed at least one week of recovery from surgery before behavioral testing began to allow full drug clearance. At the completion of testing, all animals were sacrificed by an overdose of anesthetic (Euthanasia, H. Schein). Cannula placements were verified by visual inspection: only

animals with proper cannula placements were included in the data analysis.

3. Drugs. To evaluate the involvement of specific opioid receptor subtypes in sucrose, saccharin, CMD and water deprivation-induced intake, the following centrally-administered general and selective opioid receptor antagonists were employed.

Naltrexone (Sigma Chemical Company), a general and non-selective opioid receptor antagonist was used to confirm opioid involvement in sucrose, saccharin and deprivation-induced intake, and to test opioid involvement in CMD intake. To evaluate dose-dependent effects, naltrexone at doses of 1, 5, 20, and 50 ug dissolved in normal saline (e.g., Arjune and Bodnar, 1990) was administered to determine whether its inhibitory effect was monotonic, or whether there were any dose-related or potency-related fluctuations. B-FNA (Research Biochemicals), an irreversible mu-selective opioid antagonist, does not differentiate between mu_1 and mu_2 subtypes (Takemori et al., 1981; Pasternak and Wood, 1986). B-FNA doses of 1, 5 and 20 ug were dissolved in normal saline (Arjune et al., 1990). Naloxonazine (synthesized by Dr. G.W. Pasternak), an irreversible mu_1-selective opioid antagonist was tested at doses of 10, 20 and 50 ug and dissolved in distilled water and 0.2% glacial acetic acid (Islam and Bodnar, 1990; Simone et al., 1986). If B-FNA and naloxonazine are equally effective in inhibiting a particular form of intake, one may assume mediation by the common mu_1 binding site. If B-FNA (mu_1 + mu_2) exerts inhibitory effects and naloxonazine (mu_1) fails to exert effects, one may assume mediation by the mu_2

binding site.

Nor-BNI (Research Biochemicals), a short-acting kappa antagonist was dissolved in normal saline and tested at doses of 1, 5 and 20 ug (Arjune and Bodnar, 1990). DALCE (synthesized by Dr. W.D. Bowen) a long-acting $delta_1$-selective antagonist was dissolved in 0.2 M hydrochloric acid in distilled water with the pH raised to 7.5-8.0 by adding 0.2 M sodium hydroxide and was tested at doses of 10, 20 and 40 ug (Arjune et al., 1991). Naltrindole (Research Biochemicals), a short-acting $delta_2$-selective antagonist was dissolved in 45% (w/v) aqueous 2-hydroxypropyl-beta-cyclodextrin buffer in distilled water and tested at doses of 1, 5 and 20 ug (AS Levine, personal communication).

All drugs were administered intracerebroventricularly in 10 ul volumes over 30 s through a stainless steel internal cannula (28-gauge, Plastic Products) which was connected to a Hamilton microsyringe by polyethylene tubing. All antagonists were prepared fresh, and were compared for potency relative to their corresponding vehicle control. The irreversible antagonists, B-FNA, naloxonazine and DALCE, were administered 24 h prior to the introduction of the test solution to allow development of their selective effects (Bowen et al., 1987; Hahn et al., 1982; Pasternak and Wood, 1986; Portoghese et al., 1980; Takemori et al., 1981). Pharmacological studies have shown that the complement of receptors following injections of naloxonazine and B-FNA returns to baseline levels after 96 hours (Pasternak and Woods, 1986; Takemori et al., 1981). On the behavioral level, it was

reported that body weight and food intake of the animals treated with these long acting opioid antagonists returns to baseline levels within one week after treatment (Arjune et al., 1990; Ukai and Holtzman, 1988; Mann et al.1988; Simone et al., 1985; Arjune and Bodnar, 1991). The short-acting antagonists, naltrexone, naltrindole and Nor-BNI, were administered 30 min prior to testing to maximize their antagonist effects (Portoghese et al., 1987, 1988; Takemori et al., 1988; Zukin and Zukin, 1981).

4. General procedures: To assess general and specific opioid receptor subtype antagonist effects upon the four forms of liquid intake, the animals were presented with the test solution for 1 h, and intake was measured at 5-min intervals. The 1-h exposure was selected because it allowed sufficient time for satiation, but prevented the possible development of obesity or other consequences of overeating due to exposure to the palatable diet. Since one mechanism by which general opiate antagonists have been postulated to decrease palatable food/fluid intake is by interfering with the maintenance, rather than the initiation, of ingestion (Kirkham and Blundell, 1984,1986), the recording of cumulative intakes over 5-min intervals allowed the examination of the time course and any possible shifts in drinking pattern due to specific antagonist administration. The food was present at all time during testing, however, animals did not eat during sessions. It should be noted that rats exposed to the sucrose, saccharin and CMD solutions sampled for water very infrequently through all conditions in all protocols. Therefore intake of the

particular ingestate was evaluated in each paradigm.

5. Statistical analysis: Analyses of variance for each specific time point were performed to ascertain significant differences among vehicle treatments and the different doses of each antagonist. Most significant F effects were $p < 0.0001$. Dunnett comparisons ($p < .05$) were used to discern significant differences between vehicle and individual antagonist doses at specific time points. Between subject ANOVA was used because mixed design was employed in which some animals received all conditions and some received only subset (see "Subjects" section for more details). A between subject ANOVA is the most conservative approach since subject variance and error variance due to subjects is not parceled out. This inclusion of the sources of variability makes it more difficult to achieve more significant results but is a more accurate representation of the design used in the study. Regression analyses determined the slopes, intercepts and ID_{50} for each antagonist for each of the four forms of intake to examine: a) whether differences were observed across antagonists for a particular form of intake, and b) whether differences were observed across intake situations for a particular antagonist. In order to examine sources of variance within these experiments correlations were performed which examined the relationship between basal intake of a given ingestate and the magnitude of the antagonists' effects.

CHAPTER 3. Opioid Antagonists and Water Deprivation-Induced Intake.

Introduction

The central aim of this dissertation is to evaluate the roles of specific opioid receptor antagonists upon sucrose, saccharin and CMD intake. However, it is important to determine whether any opioid effects upon intake of these solutions are due to either orosensory alterations in the perceived hedonic qualities of the solutions, or alterations in the amount of fluid intake. Since sucrose, saccharin and CMD will be presented in liquid form, it is important to determine whether any selective opioid antagonist effects were due to a decrease in fluid intake. To this end, the effects of general and specific opioid receptor subtype antagonists upon water intake in water-deprived rats were examined. Naloxone and naltrexone suppress fluid intake under both spontaneous and water deprivation conditions (e.g., Brown and Holtzman, 1979; Holtzman, 1975, 1979; Maickel et al., 1977; Millan and Morris, 1988).

Naloxone's effects are stereospecific (Brown and Holtzman, 1981; Cooper and Turkish, 1983) and are centrally-mediated (Brown and Holtzman, 1981; Czech et al., 1983; Siviy et al., 1981; Ukai and Holtzman, 1987). Opioid agonist effects appear more complex. Water intake under spontaneous and deprivation conditions is decreased by morphine (Chance and Rosecrans, 1977; Cooper, 1980, 1981; Czech et al., 1984; Sanger and McCarthy, 1980) and enkephalin analogues (DeCarro et al., 1979; Spencer et al., 1986; Summy-Long et al., 1981). In contrast, kappa-selective

agonists increase water intake under spontaneous conditions (Cooper et al., 1985; Sanger and MaCarthy, 1981; Turkish and Cooper, 1984). Based on previous studies, it was expected that naltrexone would significantly and dose-dependently inhibit water intake. Since selective opioid receptor agonists had differential effects upon water intake, it was expected that selective antagonists would show a differential pattern of effects as well.

Methods

Protocol: In each drug condition, all rats were weighed and water, but not food, was then removed from their cages for 24 h prior to testing. At 2-7 h into the light cycle, body weight and food intake were assessed and preweighed food and water (100 ml., 1 ml gradations) in a sipper tube were reintroduced. Intake of water was assessed at 5-min intervals for 1 h, and food consumption was measured after this interval. After these measurements, premeasured food and water were again presented to the rats. Food and water intake, as well as body weight were then assessed 23 h later. Food intakes were determined by weighing food pellets prior to and after each condition, and adjusting for spillage which was collected by paper under the wire mesh cage.

Each rat received between 3 to 9 microinjection conditions paired with water deprivation according to a counterbalanced order at weekly intervals. A first group of rats received: a) vehicle (n=21), b) naltrexone at doses of 1 (n=8), 5 (n=10), 20 (n=9), and 50 (n=9) ug, c) B-FNA at doses of 1 (n=13), 5 (n=13), and 20 (n=14)

ug, and d) Nor-BNI at doses of 1 (n=8), 5 (n=7), and 20 (n=8) ug. A second group of rats received: a) vehicle (n=13), b) DALCE at doses of 10 (n=7), 20 (n=7), and 40 (n=7) ug, c) naltrindole at doses of 1 (n=7), 5 (n=9), and 20 (n=9) ug, and buffer (n=8), and d) naloxonazine at doses of 10 (n=7), 20 (n=8) and 50 (n=7) ug. As indicated, naltrexone, Nor-BNI and naltrindole were administered 30 min prior to water reintroduction, whereas B-FNA, naloxonazine and DALCE were administered 24 h prior to water reintroduction.

Results

Water intake failed to differ significantly as a function of the saline vehicles (controls for naltrexone, B-FNA and Nor-BNI) and the vehicle solutes for naloxonazine and DALCE. Therefore, these vehicle values were pooled for analysis. In contrast, the buffer vehicle for naltrindole significantly reduced water intake relative to saline vehicle. Therefore, naltrindole effects were compared relative to buffer treatment.

Antagonist effects upon deprivation-induced water intake

Naltrexone significantly and dose-dependently inhibited deprivation-induced water intake at each of the 5-min intervals over the entire 1 h time course (Figure 1). Naltrexone significantly inhibited water intake over the 1 h time course following the 20 (53-67%) and 50 (40-54%) ug doses. The inhibitory effects of the 20 ug dose of naltrexone were most potent from 15-60 min after water reintroduction, and also significantly decreased water intake 24 h later by 36% (Table 1A). In contrast, the

FIGURE 1. Alterations in deprivation-induced water intake (ml) following central microinjections of vehicle (filled circle) and the general opioid antagonist, naltrexone (1 ug - open circle, 5 ug - filled square, 20 ug - open square, 50 ug filled triangle). Whereas the three higher doses of naltrexone significantly and dose dependently reduced water intake (5 ug: 38%, 20-60 min; 20 ug: 66%, 5-60 min; and 50 ug: 53%, 5-60 min), the lowest naltrexone dose (1 ug: 24%) failed to significantly inhibit water intake. Standard error range: 0.54-1.20.

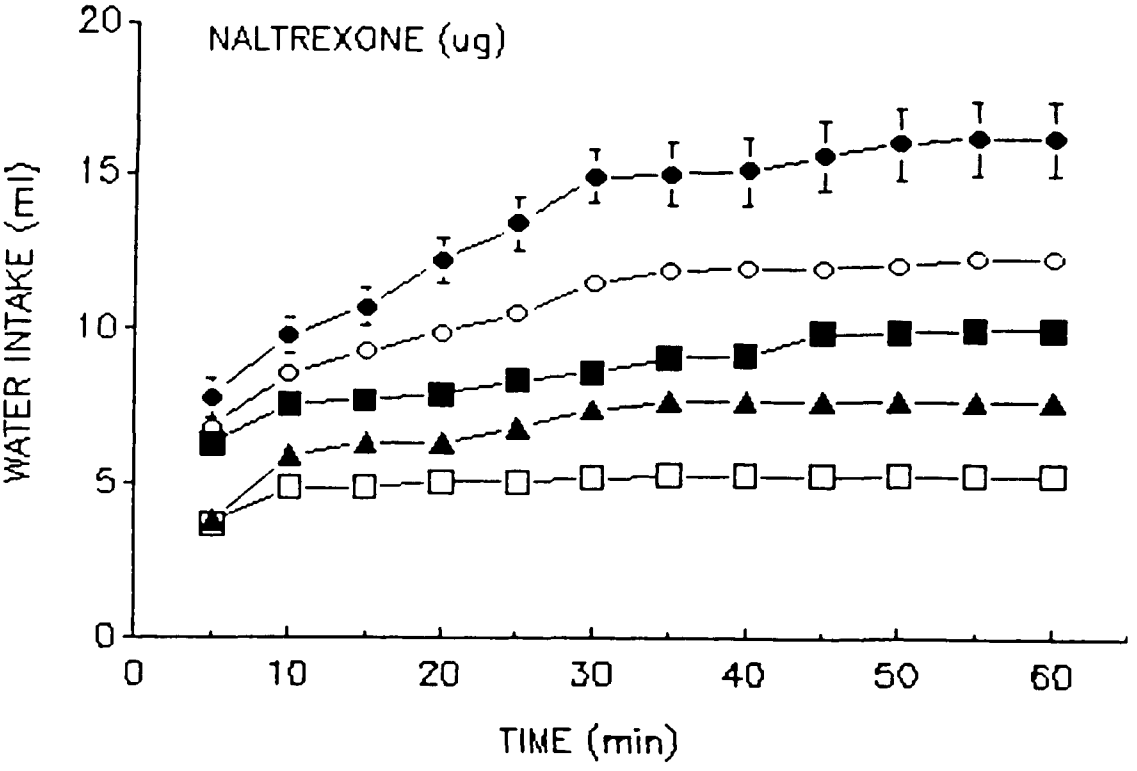

NALTREXONE (ug)
WATER INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

Table 1. Summary of opioid antagonist effects upon deprivation-induced water intake (ml) after 24 h.

A. Naltrexone (ug):

	0	1	5	20	50
Mean	60.4	51.5	51.7	38.8*	47.7
SEM	4.4	2.9	5.4	5.3	2.4

B. Nor-BNI (ug):

	0	1	5	20
Mean	60.4	68.4	58.4	47.0
SEM	4.4	4.4	8.1	5.7

C. B-FNA (ug):

	0	1	5	20
Mean	60.4	69.0	74.2	62.3
SEM	4.7	6.2	6.5	5.5

D. Naltrindole (ug):

	0	1	5	20
Mean	51.3	46.7	50.9	55.4
SEM	5.9	7.9	5.2	5.6

E. DALCE (ug):

	0	10	20	40
Mean	78.3	85.3	81.3	74.7
SEM	4.1	6.0	4.8	4.5

F. Naloxonazine (ug):

	0	10	20	50
Mean	79.9	77.4	81.3	74.7
SEM	4.1	6.3	4.6	3.9

* denotes a significant difference relative to corresponding vehicle treatment.

significant inhibition of water intake (42%) by a lower (5 ug) naltrexone dose occurred only after 20 min of exposure. Dose-dependent decreases in 1-h water intake occurred following naltrexone doses of 1 (24%), 5 (38%), 20 (66%), and 50 (52%) ug. Naltrexone's inhibition of deprivation-induced water intake was not significantly related ($r(9) = -0.31$) to the baseline intake.

Nor-BNI failed to significantly alter deprivation-induced water intake at any of the 5-min intervals (Figure 2), and failed to alter water intake 24 h after water reintroduction (Table 1B). Nor-BNI (20 ug) non-significantly reduced intake by 30-40% after 25-35 min. Decreases in 1-h water intake occurred following Nor-BNI doses of 1 (14%), 5 (11%), and 20 (28%) ug. The ability of Nor-BNI to inhibit deprivation-induced water intake was significantly related ($r(8) = -0.81$, $p < 0.05$) to the amount of baseline intake. In individual animals, Nor-BNI was more potent in inhibiting intake in rats with high baseline water intakes, and less potent in rats with lower baseline water intakes.

B-FNA significantly and dose-dependently inhibited deprivation-induced water intake relative to vehicle at each of the 5-min intake intervals over the 1-h time course (Figure 3). The inhibitory effects of B-FNA were most pronounced following the intermediate B-FNA dose (5 ug: 36-50%) in the first 30 min of the exposure. Whereas the two lower doses of B-FNA were effective across the entire time course, the highest B-FNA dose significantly inhibited deprivation-induced water intake only after 25-30 minutes. B-FNA failed to alter water intake 24 h after water

FIGURE 2. Alterations in deprivation-induced water intake (ml) following central microinjections of vehicle (filled circle) and the kappa selective opioid antagonist, Nor-BNI (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of the antagonist non-significantly reduced water intake (1 ug: 14%, 5 ug: 11%, and 20 ug: 28%). Standard error range 0.54-1.20.

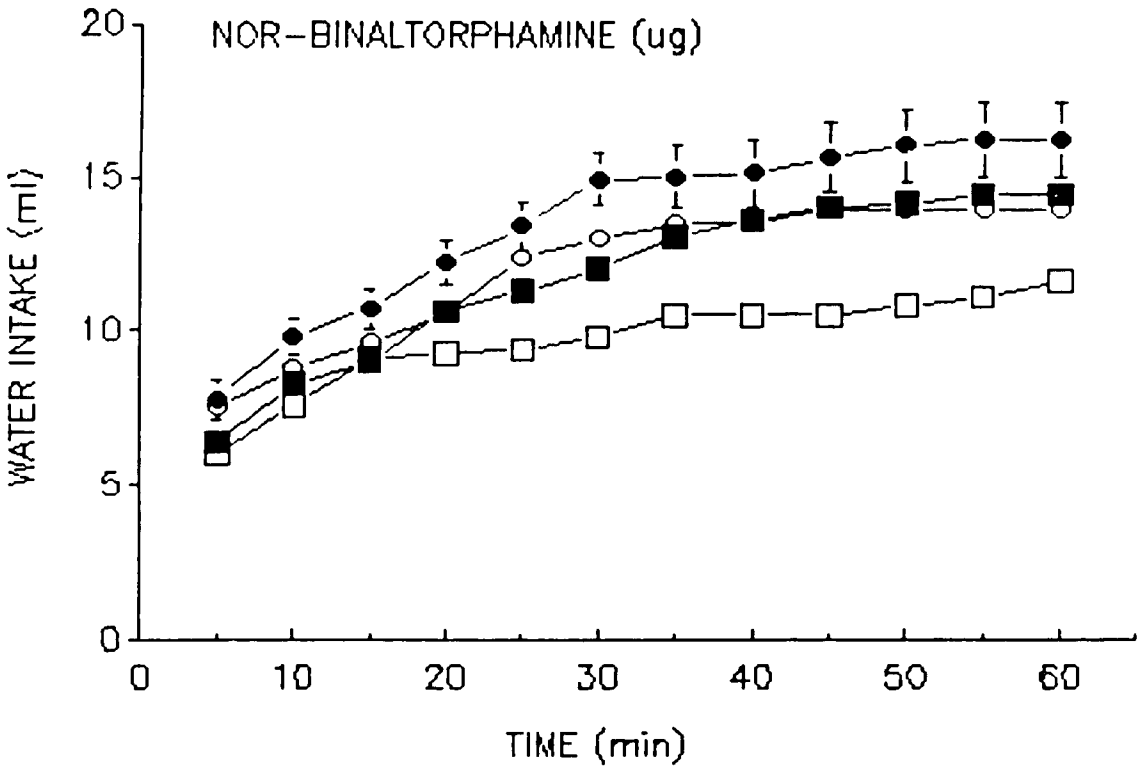
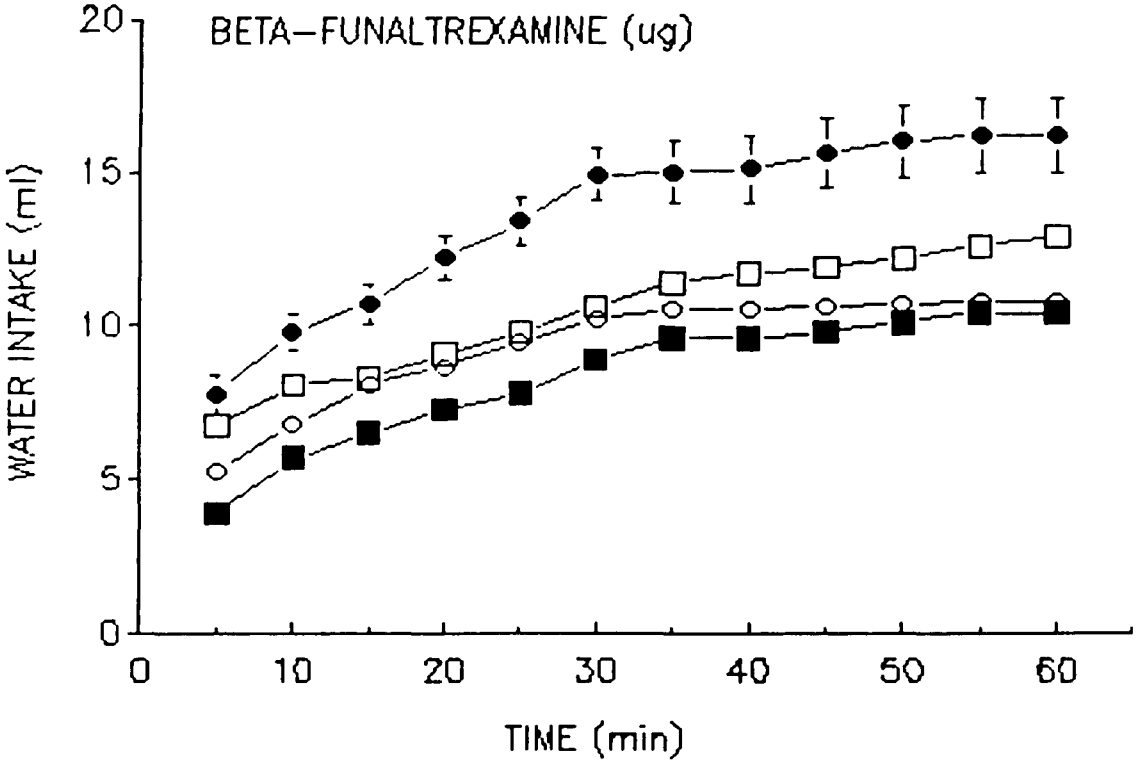

NOR-BINALTORPHAMINE (ug)
WATER INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 3. Alterations in deprivation-induced water intake (ml) following central microinjections of vehicle (filled circle) and the mu selective opioid antagonist, B-FNA (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of B-FNA significantly and dose-dependently reduced water intake (1 ug: 33%, 5-60 min; 5 ug: 35%, 5-60 min; and 20 ug: 20%, 25-55). Standard error range 0.54-1.20.


BETA–FUNALTREXAMINE (ug)
WATER INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

reintroduction (Table 1C). Decreases in 1-h water intake occurred following B-FNA doses of 1 (33%), 5 (36%), and 20 (20%) ug. B-FNA's inhibition of deprivation-induced water intake was not significantly related ($r(14) = -0.25$) to baseline intake.

Naltrindole failed to significantly alter deprivation-induced water intake relative to the vehicle-buffer at any of the 1 h intake intervals (Figure 4), or at 24 h after water reintroduction (Table 1D). Naltrindole non-significantly stimulated water intake following the 1 (2-29%) and 20 (6-14%) ug doses with the effect increasing over the time course. In contrast, naltrindole (5 ug) non-significantly inhibited water intake by 35% after 5 to 20 min. Naltrindole's failure to alter deprivation-induced water intake could not be explained by differences in baseline intakes across animals ($r(9) = 0.18$).

DALCE failed to significantly alter deprivation-induced water intake at any of the 1 h intake intervals (Figure 5), or at 24 h after water reintroduction (Table 1E). DALCE non-significantly stimulated water intake following the 10 (3-20%) and 20 (8-28%) ug doses with the effect strongest at onset. DALCE (40 ug) non-significantly stimulated intake after 15-25 min (1-13%), and then inhibited intake after 30-60 min (10-12%). Although there was a significant inverse relationship between baseline intake and DALCE-induced effects ($r(7) = -0.73$, $p < 0.05$), evaluation of the individual effects indicated that DALCE never produced appreciable inhibition of intake. Naloxonazine failed to significantly alter deprivation-induced water intake at any of the 1 h intake intervals (Figure 6), or

FIGURE 4. Alterations in deprivation-induced water intake (ml) following central microinjections of vehicle buffer (filled circle) and the $delta_2$ selective opioid antagonist, naltrindole (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of naltrindole non-significantly altered water intake (1 ug: 29% increase, 5 ug: 16% decrease, and 20 ug: 13% increase) but failed to reach significance. Standard error range 0.75-1.86.

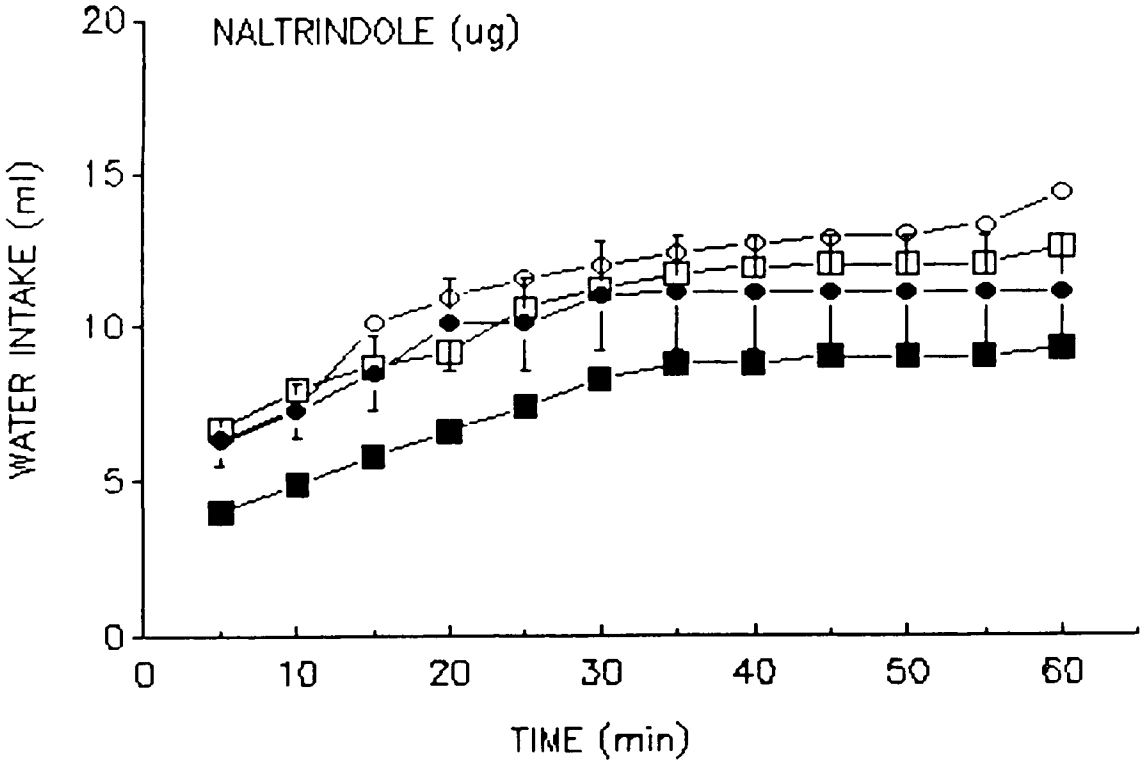
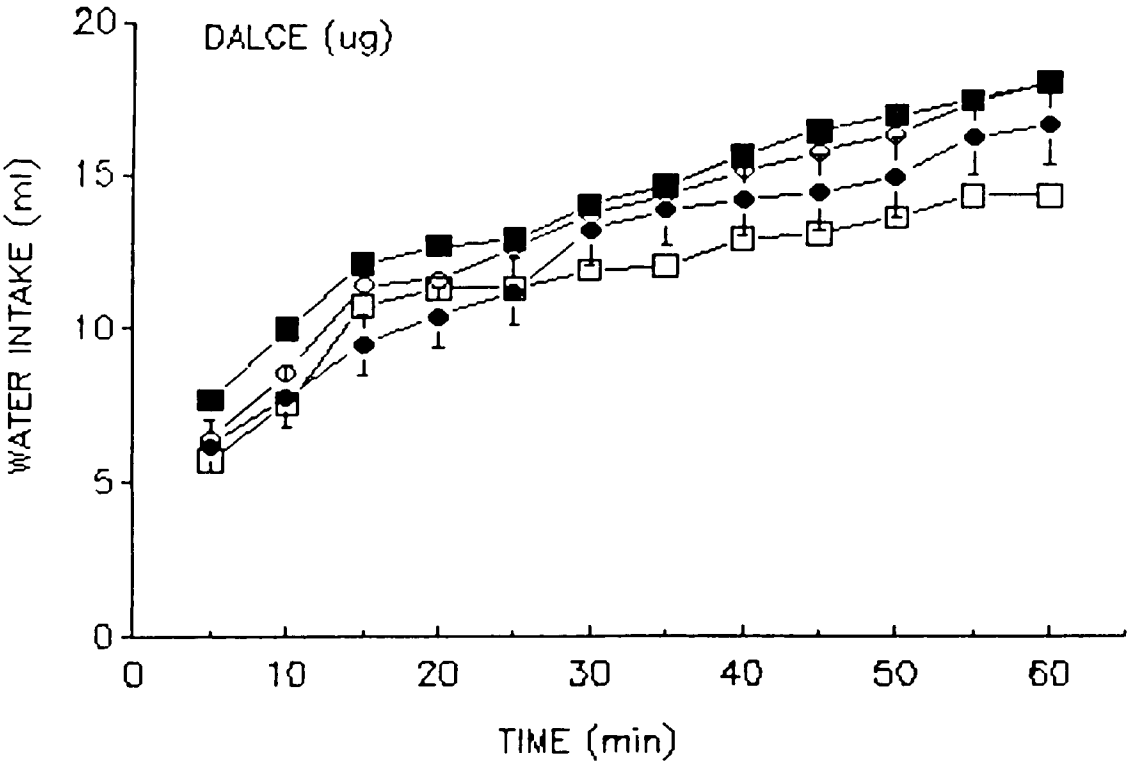

NALTRINDOLE (ug)
WATER INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 5. Alterations in deprivation-induced water intake (ml) following central microinjections of vehicle (filled circle) and the $delta_1$ selective opioid antagonist, DALCE (10 ug - open circle, 20 ug - filled square, 40 ug - open square). All three doses of DALCE non-significantly altered water intake (10 ug: 8% increase, 20 ug: 8% increase, and 40 ug:12% reduction). Standard error range 0.81-1.33.


DALCE (ug)
WATER INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

24 h after water reintroduction (Table 1F). Naloxonazine non-significantly stimulated water intake following the 10 (3-37%), 10-50 min), 20 (1-27%, 10-45 min) and 50 (8-14%, 5-25 min) ug doses with the effect strongest at onset. Although there was a significant inverse relationship between baseline intake and naloxonazine-induced effects ($r(7)=-0.81$, $p < 0.05$), evaluation of the individual effects indicated that naloxonazine never produced appreciable inhibition of intake.

Antagonist effect upon food intake following water deprivation.

Naltrexone significantly reduced food intake by 70% after 1 h of water reintroduction, but not after 24 h of water reintroduction (Table 2A). Nor-BNI significantly reduced food intake by over 90% after 1 h of water reintroduction, but not after 24 h of water reintroduction (Table 2B). B-FNA significantly and dose-dependently reduced food intake during water deprivation, which is in keeping with its effects upon food deprivation (Arjune et al., 1990; Levine et al., 1991). B-FNA also significantly reduced food intake by 68% after 1 h of water reintroduction, but not after 24 h of water reintroduction (Table 2C). Naltrindole failed to alter food intake at 1 or 24 h after water reintroduction (Table 2D). The reductions in food intake after 1 (64%) and 24 (20%) h of water reintroduction following the high dose of DALCE approached, but did not achieve significance (Table 2E). The low dose of DALCE, significantly reduced food intake during water deprivation, an effect similar to that following food deprivation (Arjune et al., 1991). Although naloxonazine failed to alter food intake after 1 h of water deprivation, it significantly

FIGURE 6. Alterations in deprivation-induced water intake (ml) following central microinjections of vehicle (filled circle) and the mu_1 selective opioid antagonist, naloxonazine (10 ug - open circle, 20 ug - filled square, 50 ug - open square). All three doses of naloxonazine non-significantly reduced water intake (10 ug: 8%, 20 ug: 10%, and 50 ug: 17%). Standard error range 0.81-1.28.

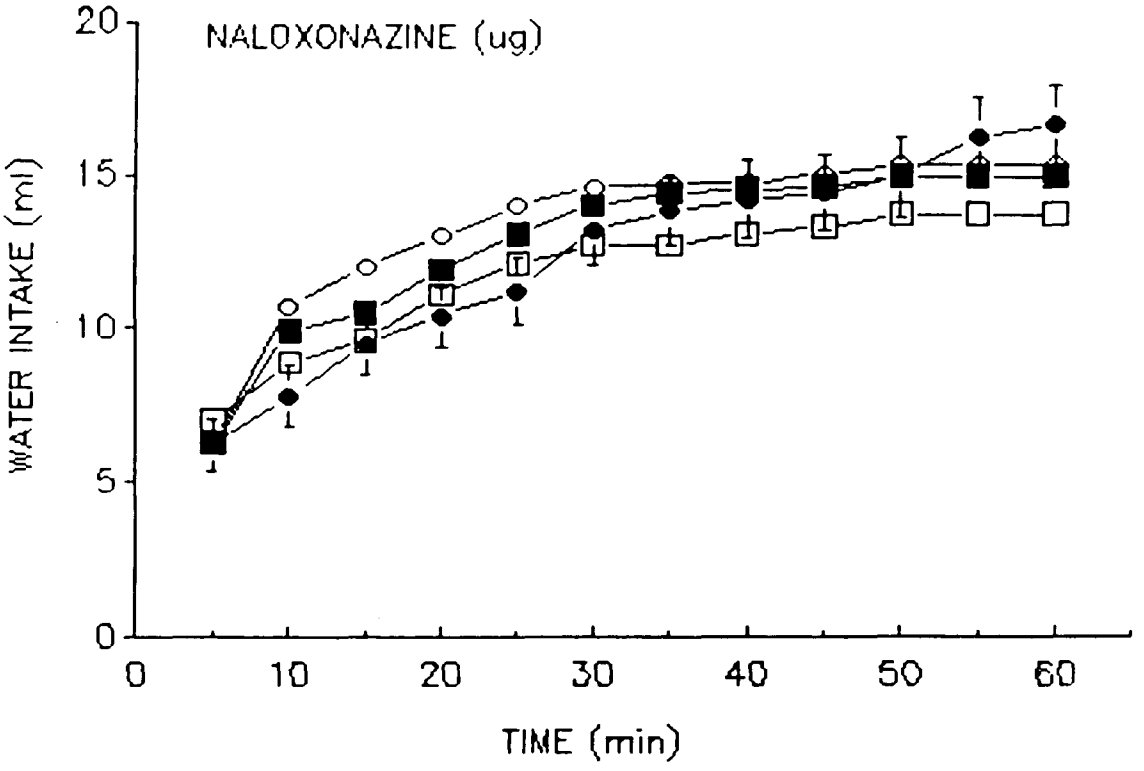

NALOXONAZINE (ug)
WATER INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

Table 2. Summary of opioid antagonist effects upon food intake (g) following water deprivation.

A. Naltrexone (ug):

	0	1	5	20	50
1h	2.4	2.0	1.5	0.7*	0.8*
24 h	22.1	22.1	20.8	15.2	20.0

B. Nor-BNI (ug):

	0	1	5	20
1 h	2.4	2.0	1.6	0.1*
24 h	22.1	24.4	16.6	21.8

C. B-FNA (ug):

	0	1	5	20
1 h	2.4	1.3	1.4	0.9*
24 h	22.1	25.4	22.9	22.4

D. Naltrindole (ug):

	0	1	5	20
1 h	1.8	2.6	1.7	1.6
24 h	18.5	20.7	21.3	21.3

E. DALCE (ug):

	0	10	20	40
1 h	2.5	2.1	2.1	0.9
24 h	27.0	25.9	27.6	21.4

F. Naloxonazine (ug):

	0	10	20	50
1 h	2.5	1.1	1.8	2.3
24 h	27.0	21.1	23.6	24.0

* denotes a significant difference relative to corresponding vehicle treatment.

decreased food intake after 24 h of water deprivation by 23% (Table 2F). Naloxonazine also significantly decreased food intake during water deprivation, in keeping with its effects following food deprivation (Simone et al., 1985).

Discussion

Central administration of naltrexone significantly and dose-dependently reduced deprivation-induced water intake across the 1-h time course. These results are in agreement with previously reported effects of the general opioid antagonists, naloxone and naltrexone, upon fluid intake (Brown and Holtzman, 1979; Holtzman, 1975, 1979; Maickel et al., 1977; Millan and Morris, 1988). The peak inhibition of water intake by naltrexone did not occur until 15-25 min after reintroduction of water. These data appear to agree with the hypothesis suggesting opioid mediation of the maintenance, rather than the initiation of intake.

Water intake under spontaneous and deprivation conditions is decreased by morphine, a prototypical mu agonist, (Chance and Rosecrans, 1977; Cooper, 1980, 1981; Czech et al., 1984; Sanger and McCarthy, 1980) and enkephalin analogues (DeCarro et al., 1979; Spencer et al., 1986; Summy-Long et al., 1981). Ukai and Holtzman (1988) found that intrahypothalamic administration of the mu-selective opioid agonist, DAMGO, increased spontaneous water intake, but decreased intake following water deprivation. Free feeding and deprivation-induced feeding were significantly reduced by B-FNA, a mu selective antagonist which does not differentiate between mu_1 and mu_2 receptor subtypes, and naloxonazine, a mu_1

selective antagonist, (Arjune et al., 1990; Simone et al., 1985; Levine et al., 1991) suggesting mu_1 mediation. In the present study, the reductions in food intake (24 h) following administration of B-FNA and naloxonazine were in agreement with previous reports (Arjune et al., 1990; Simone et al., 1985) supporting a mu_1 opioid mediation of free feeding. In contrast, the present study illustrated that water-deprivation induced intake was inhibited by B-FNA but it was not affected by naloxonazine. The ability of B-FNA and the inability of naloxonazine to antagonize water intake indicate a role for the mu_2 receptor subtype in these effects. Insulin and 2DG hyperphagia were also shown to be inhibited by blockade of the mu_2 receptor (Beczkowska and Bodnar, 1991; Arjune and Bodnar, 1990). It seems that involvement of this opioid receptor subtype is prominent in certain challenge situations.

Kappa-selective agonists increase water intake under spontaneous conditions (Cooper et al., 1985; Sanger and MaCarthy, 1981; Turkish and Cooper, 1984), but intrahypothalamic administration of the kappa-selective agonist, dynorphin $A_{(1-13)}$, failed to alter deprivation-induced water intake (Ukai and Holtzman, 1988). Hyperphagia following food deprivation was only marginally decreased by kappa-selective Nor-BNI (Arjune et al., 1991; Levine et al., 1990). In the present study, Nor-BNI significantly reduced food intake during the 1 h drinking session, had no effect upon food intake 24 h following water reintroduction, and it failed to significantly alter deprivation-induced water intake. Nor-BNI's effects upon food

intake seem to be in agreement with previous reports (Arjune et al., 1991). A significant correlation between the magnitude of Nor-BNI's effects and baseline intake suggests an additional explanation with respect to the role of kappa receptor in water intake. Since the kappa blockade was very potent in inhibiting the water intake of animals with high baseline intakes in response to water deprivation, but less effective in reducing water intakes of animals with low deprivation-induced baseline intakes, this suggests that the kappa receptor may be activated only after the intake exceeds a certain level. This suggests that some other opioid receptor subtypes (perhaps mu_2) may modulate water intake up to a certain level, and then the kappa receptor is "turned on" to modulate further intake. Hence, very little inhibition would be observed in animals with low baseline intake following administration of Nor-BNI because the kappa receptor has not been activated yet. It is possible that this dichotomy might also explain the marginal effectiveness of Nor-BNI in reducing hyperphagia following food deprivation. It would be of interest to determine whether kappa blockade would inhibit food intake in animals with high food intake but fail to reduce hyperphagia in animals with low food intake.

Water intake under spontaneous and deprivation conditions is decreased by enkephalin analogues (DeCarro et al., 1979; Spencer et al., 1986; Summy-Long et al., 1981), however, intrahypothalamic injections of delta-selective agonist, DPDPE, failed to alter water intake (Ukai and Holtzman, 1988). In addition, neither DALCE ($delta_1$) nor naltrindole ($delta_2$) altered deprivation-induced food intake (Arjune et

al., 1991; AS Levine, personal communication). In the present study, naltrindole failed to alter food intake, and the reductions following the high dose of DALCE approached, but did not achieve significance. The low dose of DALCE significantly reduced food intake during water deprivation, an effect similar to that following food deprivation (Arjune et al., 1991). Both of the delta antagonists failed to significantly inhibit deprivation-induced water intake, although, there was a significant negative correlation between the inhibitory effects of DALCE and the baseline intake of the animals.

Water deprivation can affect the level of opioid receptors. This is a potential confounding variable since long acting opioid antagonists were given at the onset of deprivation, while short-acting opioid antagonists were given after 23 hours of deprivation. Therefore, deprivation-induced changes in opioid function occurred after long-term antagonism but before administration of the short-term antagonists. If these deprivation-induced opioid effects were responsible for the antagonistic effects one would expect homogenous alterations by either all short-term opioid antagonists or all long-term antagonists. This was clearly not the case. Whereas, the short-term opioid antagonist naltrexone decreased deprivation-induced drinking the other short-term opioid antagonists, Nor-BNI and naltrindole did not. Further, whereas B-FNA, a long-acting antagonist, reduced deprivation-induced intake, the other long-term antagonists, naloxonazine and DALCE did not. Since only naltrexone and B-FNA significantly reduced deprivation-induced water intake, one

might expect that B-FNA should exert equal effects to the general opiate antagonist. However, naltrexone exerted greater inhibitory effects upon deprivation-induced water intake than B-FNA, which suggests that a second or third opioid receptor subtype might participate with the mu_2 receptor in inhibiting deprivation-induced water intake. Based on the observations reported above it seems that the kappa receptor might play a secondary role in regulation of deprivation-induced water intake. A future approach using multiple antagonists administration might clarify which subtype combinations produce maximal opioid inhibitory effects.

In addition, deprivation paradigm was employed to control for the amount of fluid consumed as compared to the taste factors in other experimental manipulations, e.g. sucrose, saccharin and CMD. It should not be inferred that palatable per se is being controlled because water deprivation may make water palatable.

CHAPTER 4. Opioid Antagonists and Sucrose Intake.

Introduction

Endogenous opioid function was initially implicated in modulating intake of sucrose based upon the ability of the general opioid receptor antagonists, naloxone and naltrexone, to reduce intake of this solution (Cooper, 1983; LeMagnen et al., 1980; Lynch, 1986; Lynch and Libby, 1983; Sclafani et al., 1982; Siviy and Reid, 1983). A further line of experiments has shown that opiate receptor antagonists reduced the preference for sweet solutions in two-bottle tests (LeMagnen et al., 1980). The antagonists may have acted, therefore, to reduce the reward which normally derives from the palatable, sweet taste (Cooper et al., 1985).

Rats with gastric fistulas also display significant reductions in intake of sweet solutions following naloxone (Kirkham and Cooper, 1988a,b; Rockwood and Reid, 1982). The antidipsogenic effects of naloxone appear to be more potent in inhibiting sweet fluid intake than water intake (Levine et al., 1982). Kirkham and Cooper (1988a,b) suggest that naloxone's inhibition of sucrose intake is most probably due to its attenuation of palatability and not postingestive factors. The present experiment aims to differentiate which of the opioid receptor subtype(s) are involved in opioid modulation of sucrose solution intake, and to discern the time course of effects of each of the selective antagonists upon sucrose intake. If opioid modulation of sucrose intake is due to its involvement in regulation of fluid intake per se, then a similar pattern of results to that of water deprivation would be expected. If

however, the opioid involvement in modulation of sucrose intake is due to factors other than the mere regulation of fluid intake, then the pattern of antagonist effects should differ.

Methods

Protocol: A 10% sucrose (Sigma Chemical Company) solution (100 g sucrose/ 1 l water) was prepared fresh before testing with tap water and sucrose crystals. To introduce the solution rats were water-deprived for 24 h, and given access to a 10 % sucrose solution (55 ml, 1 ml gradations) in a sipper tube attached to the front of the cage for 1 h. Rats were tested at three subsequent ad libitum occasions in which they had access to the 10% sucrose solution in the front of the cage, and their automatic watering system at the rear of the cage. Individual intakes were assessed over 1 h, and a criterion sucrose intake of at least 10 ml was necessary for the rat to continue in the paradigm.

Each rat received up to 9 microinjection conditions according to a counterbalanced order at weekly intervals at 2-7 h into the light cycle. A first group of rats received: a) vehicle (n=18), b) naltrexone at doses of 1 (n=6), 5 (n=6), 20 (n=6), and 50 (n=12) ug, c) B-FNA at doses of 1 (n=6), 5 (n=6) and 20 (n=6) ug, and d) Nor-BNI at doses 1 (n=6), 5 (n=6) and 20 (n=6) ug. A second group of rats received: a) vehicle (n=16), b) DALCE at doses of 10 (n=7), 20 (n=8) and 40 (n=10) ug, c) naltrindole at doses of 1 (n=8), 5 (n=7) and 20 (n=9) ug, and buffer (n=8), and d) naloxonazine at doses of 10 (n=8), 20 (n=8), and 50 (n=9) ug.

Sucrose intake was assessed at 5-min intervals for 1 h and cumulative intake was recorded. To determine whether each rat recovered its baseline sucrose intake, an injection-free sucrose drinking session occurred 48-72 h after each drug condition. All rats in this paradigm had to recover their baseline intakes to continue in the study.

Results

Naltrexone significantly and dose-dependently inhibited sucrose intake at each of the 5-min intake intervals over the 1-h time course (Figure 7). Relative to vehicle, the highest dose of naltrexone inhibited sucrose intake across the entire time course, whereas the three lowest doses inhibited sucrose intake after 10 min of exposure. The peak inhibitory effect (65%) of the highest naltrexone dose occurred at 10-30 min after sucrose exposure. Decreases in overall sucrose intake occurred following naltrexone doses of 1 (31%), 5(34%), 20 (53%), and 50 (53%) ug. Naltrexone's inhibition of sucrose intake was not significantly related ($r(6)=-0.44$) to baseline intake.

Nor-BNI significantly and dose-dependently inhibited sucrose intake at all intake intervals from 10-60 min (Figure 8). Relative to vehicle, the two higher Nor-BNI doses significantly inhibited sucrose intake after 10 min, whereas the lowest dose significantly inhibited sucrose intake after 25 min. The peak inhibitory effect (55%) of the highest Nor-BNI dose occurred 15-60 min after sucrose exposure. Decreases in overall sucrose intake occurred following Nor-BNI doses of 1 (30%), 5 (50%), and

FIGURE 7. Alterations in 10% sucrose solution intake (ml) following central microinjections of vehicle (filled circle) and the general opioid antagonist, naltrexone (1 ug - open circle, 5 ug - filled square, 20 ug - open square, 50 ug - filled triangle). All four doses of naltrexone significantly and dose-dependently inhibited sucrose intake (1 ug: 31%, 10-60 min; 5 ug: 34%, 10-60 min; 20 ug: 53%, 10-60 min; and 50 ug: 53%, 5-60 min). Standard error range 0.98-1.36.

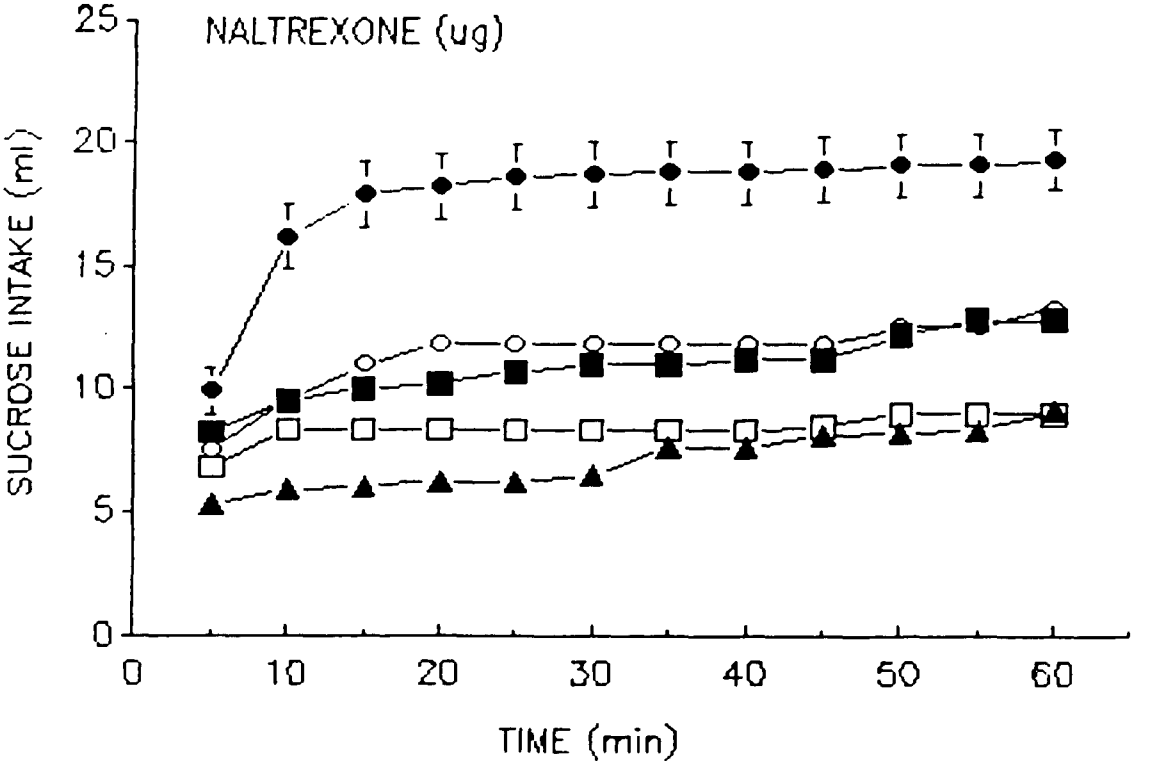
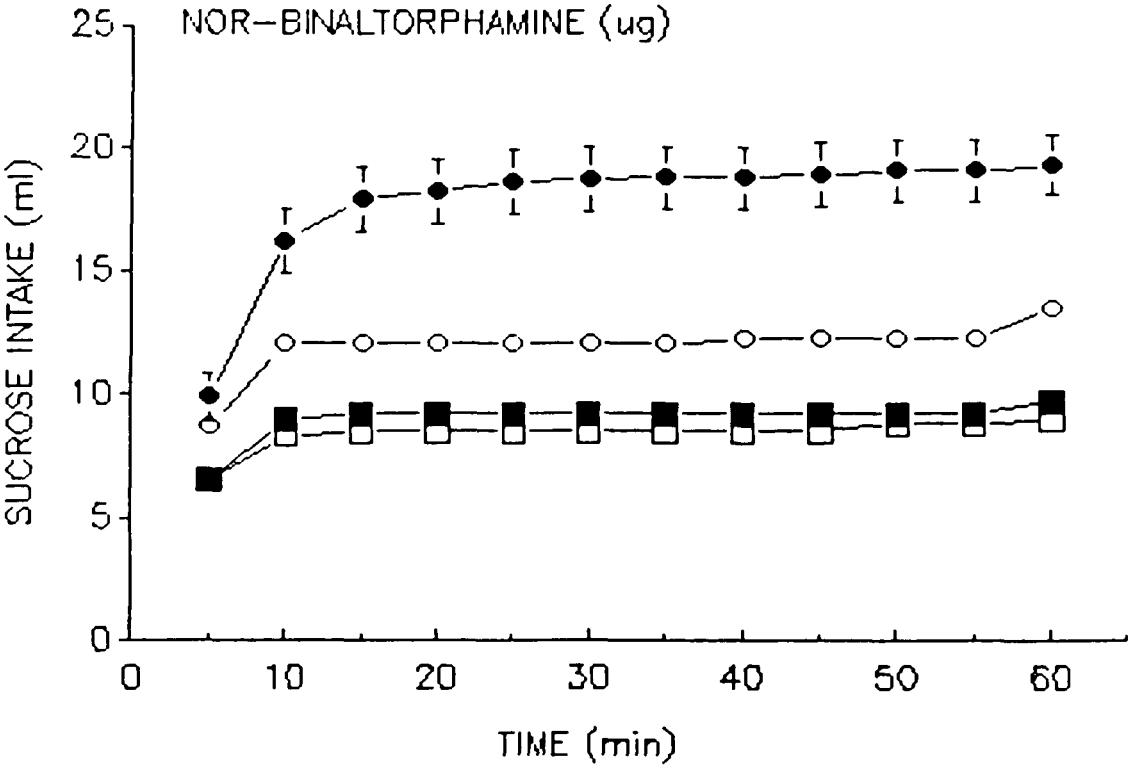

NALTREXONE (ug)
SUCROSE INTAKE (ml)
TIME (min)
0
5
10
15
20
25
0
10
20
30
40
50
60

FIGURE 8. Alterations in 10% sucrose solution intake (ml) following central microinjections of vehicle (filled circle) and the kappa-selective opioid antagonist, Nor-BNI (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of Nor-BNI significantly and dose-dependently inhibited sucrose intake (1 ug: 30%, 25-55 min; 5 ug: 50%, 10-60 min; and 20 ug: 53%, 10-60 min). Standard error range 0.98-1.36.


NOR-BINALTORPHAMINE (ug)
SUCROSE INTAKE (ml)
25
20
15
10
5
0
0
10
20
30
40
50
60
TIME (min)

A significant relationship between baseline intake and Nor-BNI-induced inhibition occurred ($r(6)=0.78$, $p=0.05$), such that Nor-BNI was most effective in reducing sucrose intake in rats with low baseline sucrose intakes.

B-FNA significantly and dose-dependently inhibited sucrose intake at all intake intervals from 10-60 min (Figure 9). Relative to vehicle, the high dose of B-FNA significantly inhibited sucrose intake after 25 min whereas the low and intermediate B-FNA doses reduced intake after 10 and 15 min respectively. The peak inhibitory effects of B-FNA (5 ug, 36%) occurred 15-60 microinjections of vehicle minutes after sucrose exposure. Decreases in overall sucrose intake occurred following B-FNA doses of 1 (16%), 5(32%), and 20 (32%) ug. B-FNA's inhibition of sucrose intake was not significantly related ($r(6)=-0.02$) to baseline intake.

Since the buffer solution for naltrindole significantly reduced sucrose intake by 20% relative to a distilled water vehicle, the effects of naltrindole were evaluated relative to this buffer. Naltrindole failed to significantly alter sucrose intake at any interval (Figure 10). Indeed, naltrindole non-significantly increased sucrose intake following the 1 (28-50%) and 5 (1-22%) ug doses with the effects most pronounced at the beginning of the drinking session. In contrast, naltrindole (20 ug) non-significantly decreased sucrose intake by 24-60% with these inhibitory effects most potent at the beginning of the drinking session. Naltrindole's failure to significantly alter sucrose intake could not be explained by differences in baseline intakes across animals ($r(9)=-0.19$).

FIGURE 9. Alterations in the intake of 10% sucrose solution (ml) following central 20 (53%) ug. (filled circle) and the mu-selective opioid antagonist, B-FNA (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of B-FNA significantly inhibited sucrose intake (1 ug: 43%, 5 min; 5 ug: 32%, 15-60 min; and 20 ug: 32%, 25-60 min). Standard error range 0.98-1.36.

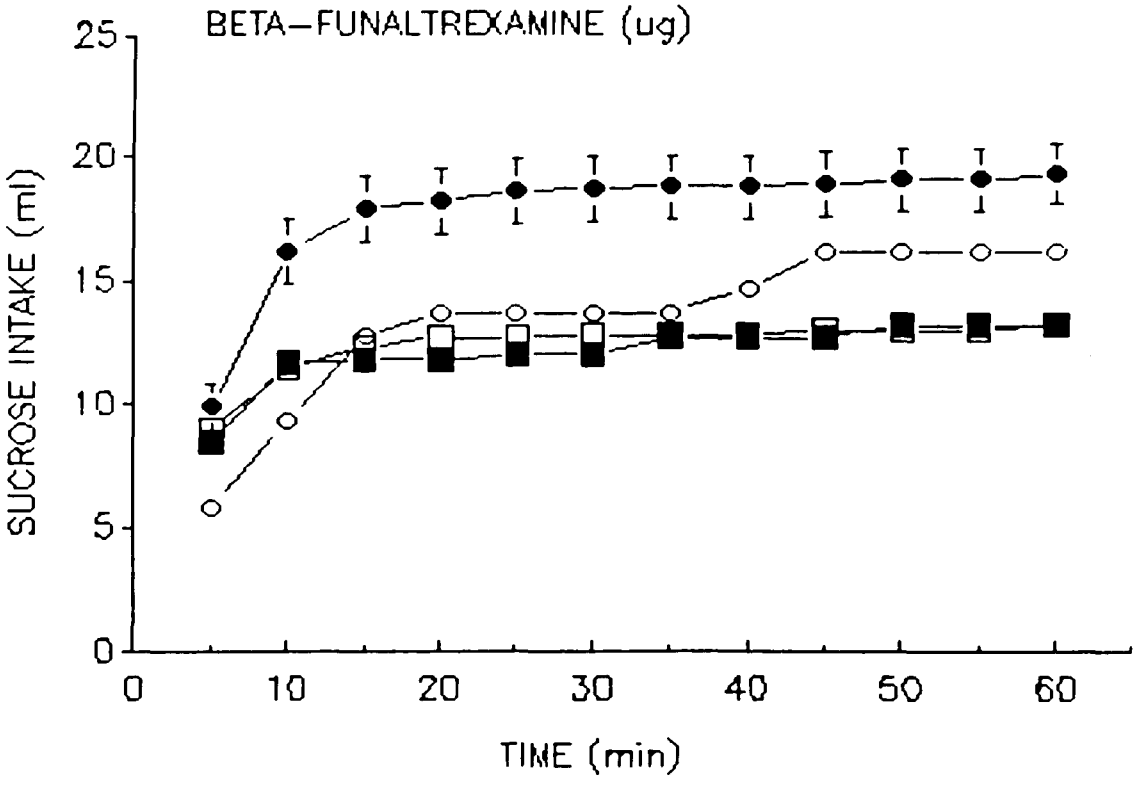
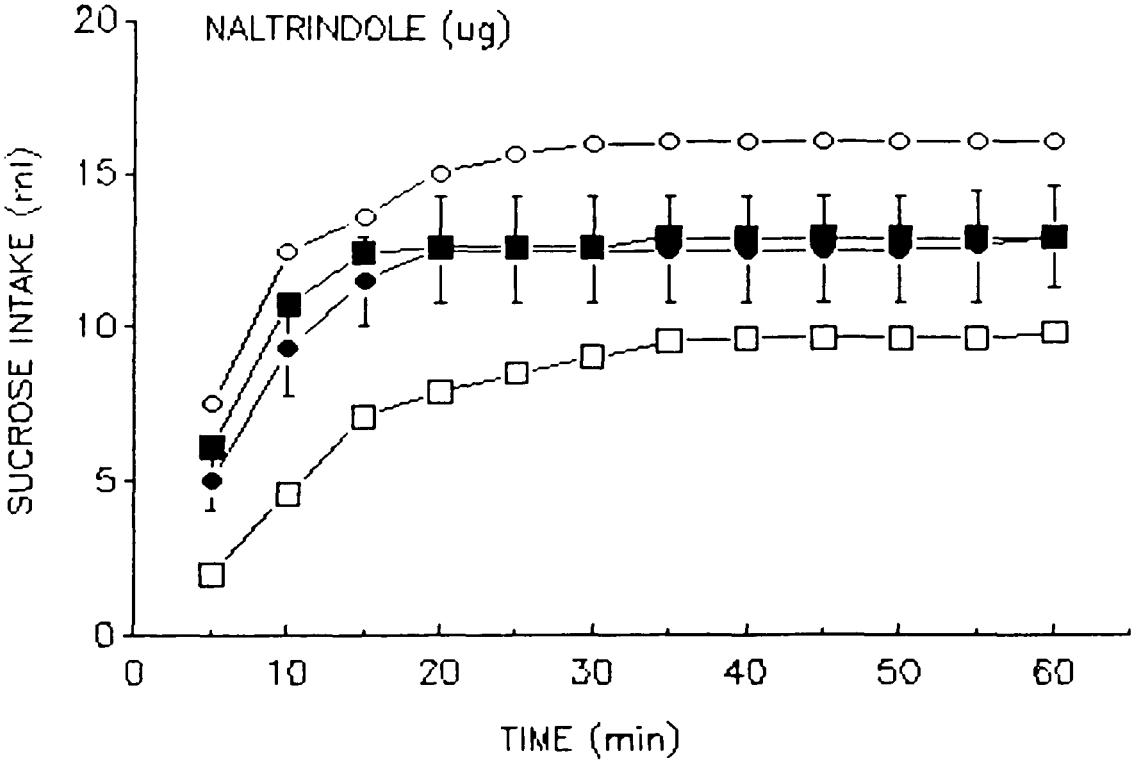

BETA–FUNALTREXAMINE (ug)
SUCROSE INTAKE (ml)
25
20
15
10
5
0
0
10
20
30
40
50
60
TIME (min)

FIGURE 10. Alterations in intake of 10% sucrose solution (ml) following central microinjections of vehicle buffer (filled circle) and the delta$_2$-selective opioid antagonist, naltrindole (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of naltrindole non-significantly altered sucrose intake: 1 ug (28% increase), 5 ug (no change) and 20 ug (24% decrease). Standard error range 0.96-1.82.


NALTRINDOLE (ug)
SUCROSE INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

DALCE significantly reduced sucrose intake only at 15 and 20 min after exposure (Figure 11) with the 20 ug dose (32-35%) effective at these intervals. In contrast, DALCE (10 ug) non-significantly stimulated sucrose intake (13-75%) with the effects most pronounced at the beginning of the drinking session. DALCE's inhibition of sucrose intake was not significantly related ($r(10) = -0.41$) to baseline intake.

Naloxonazine significantly increased sucrose intake only at 5 and 10 min after exposure (Figure 12) with the 10 ug dose (64-134%) effective at these intervals. This dose subsequently increased sucrose intake (26-35%) non-significantly. Naloxonazine (20 ug) non-significantly stimulated sucrose intake (4-32%) with the effects dissipating over time. Naloxonazine (50 ug) non-significantly decreased sucrose intake (21%) over the 1-h time course. Naloxonazine's alterations of sucrose intake were not significantly related ($r(9) = -0.37$) to baseline intake.

Discussion

Central administration of both naltrexone and the selective kappa receptor subtype antagonist, Nor-BNI significantly and dose-dependently reduced sucrose intake across the 1-h time course. Central administration of the selective mu receptor subtype antagonist, B-FNA, significantly reduced sucrose intake over the 1-h time course to a smaller degree. In contrast, central administration of the selective mu_1 receptor antagonist, naloxonazine, transiently stimulated sucrose intake. Whereas the selective $delta_1$ receptor antagonist, DALCE, transiently

FIGURE 11. Alterations in intake of 10% sucrose solution (ml) following central microinjections of vehicle (filled circle) and the $delta_1$-selective opioid antagonist, DALCE (10 ug - open circle, 20 ug - filled square, 40 ug - open square). The middle dose of DALCE significantly inhibited sucrose intake (20 ug: 35%, 15-20 min). Overall sucrose intake was non-significantly stimulated by the 10 ug dose (13%), and was reduced by the 20 (28%) and 40 (24%) ug dose of DALCE. Standard error range 0.48-1.50.

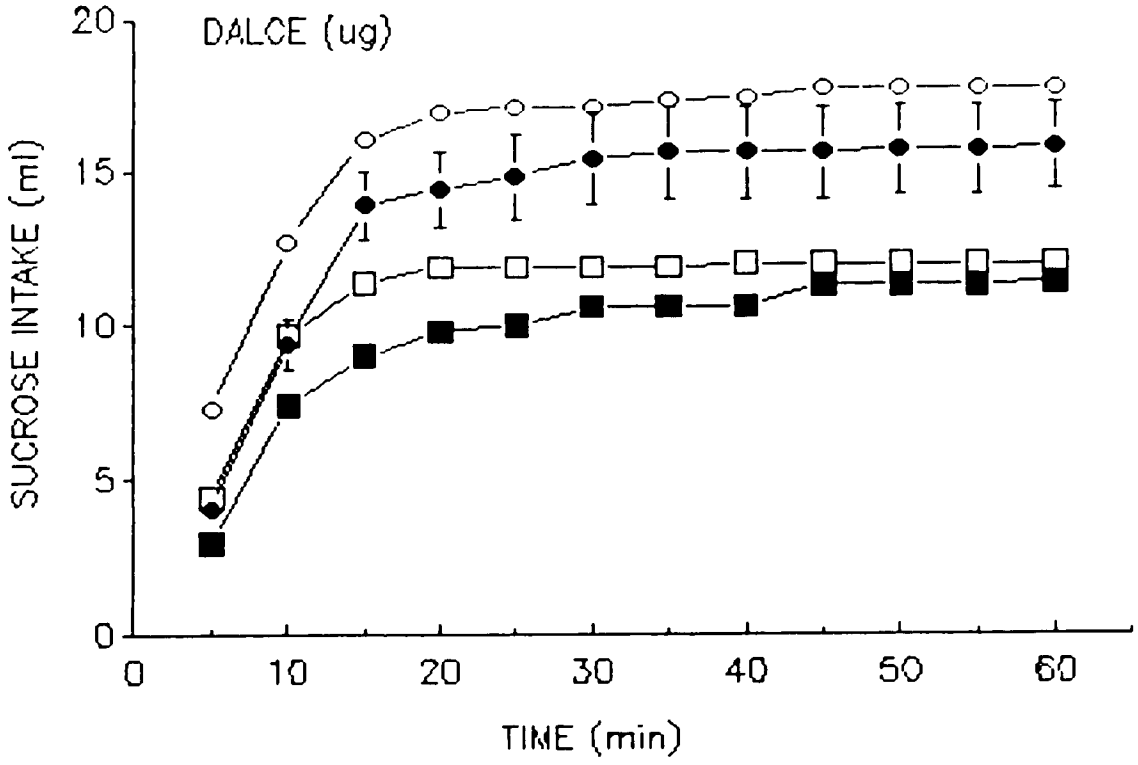
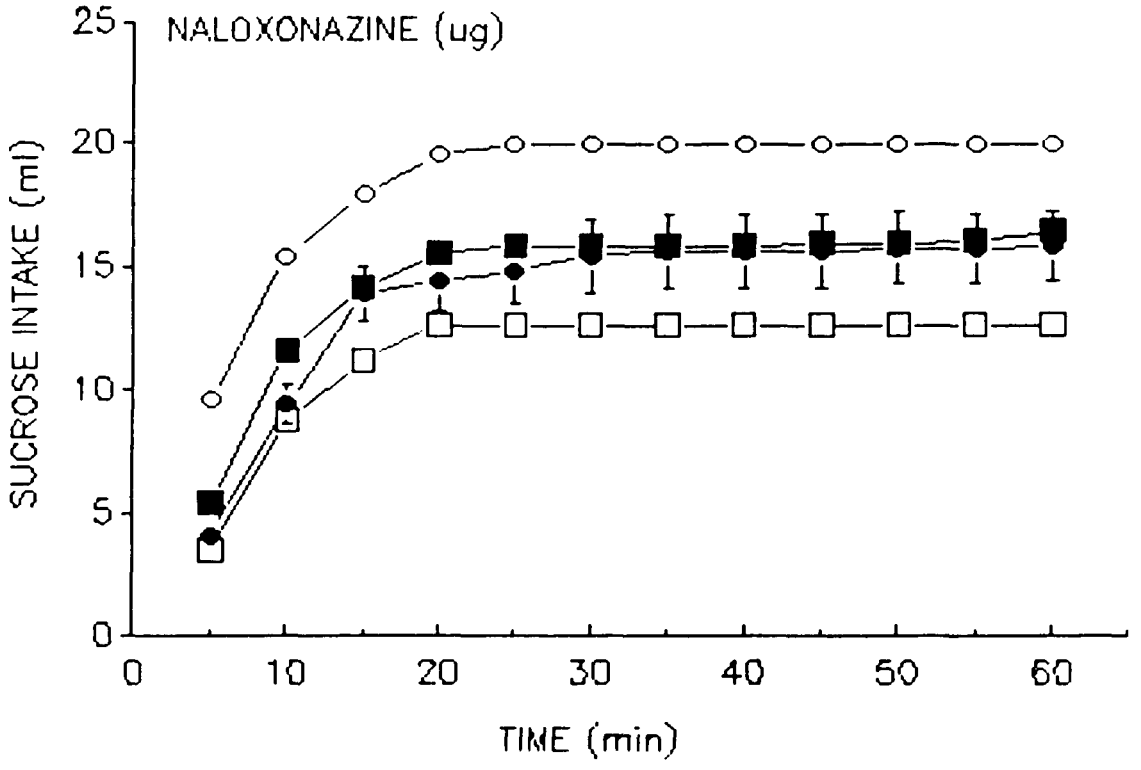

DALCE (ug)
SUCROSE INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 12. Alterations in intake of 10% sucrose solution (ml) following central microinjections of vehicle (filled circle) and the mu_1-selective opioid antagonist, naloxonazine (10 ug - open circle, 20 ug - filled square, 50 ug - open square). The low naloxonazine dose significantly stimulated sucrose intake (10 ug: 64-134%, 5-10 min). The overall sucrose intake was non-significantly stimulated by 10 (26%) and 20 (4%) ug doses of naloxonazine, and was non-significantly reduced by the 50 (21%) ug dose of naloxonazine. Standard error range 0.48-1.50.


NALOXONAZINE (ug)
SUCROSE INTAKE (ml)
TIME (min)
0
5
10
15
20
25
0
10
20
30
40
50
60

decreased sucrose intake, the selective delta$_2$ receptor antagonist, naltrindole, failed to significantly alter sucrose intake. These data suggest that the kappa and mu$_2$ opioid binding sites are involved in the opioid mediation of sucrose intake.

Sucrose intake in non-deprived rats appears to be a model of palatable aspects of ingestion. This sugar has a very strong oral characteristic as well as postingestive consequence. It has been suggested that post-ingestive effects of sucrose, and other palatable substances, appear to modulate the orosensory perception of palatability (Sclafani, 1991). A role for endogenous opioids in modulating this response has been based upon the ability of general opioid antagonists to reduce sucrose intake (e.g. Kirkham and Cooper, 1988a,b). A role for endogenous opioids in modulating the palatable aspects of sucrose intake is further supported by three observations: a) naloxone reduces sucrose intake in rats with gastric fistulas which eliminates the post-ingestive consequences of the ingestate (Kirkham and Cooper, 1988a,b; Rockwood and Reid, 1982), b) sucrose intake is more sensitive to the antagonistic properties of naloxone than water intake (Levine et al., 1982), and c) mu-selective and delta-selective opioid agonists stimulate intake of sweet solutions (Gosnell and Majchrzak, 1989). One mechanism by which general opiate antagonists have been postulated to decrease intake is by interfering with the maintenance, rather than the initiation, of feeding (Kirkham and Blundell, 1984; 1986). In the sham feeding preparation, Kirkham and Cooper (1988a,b) observed that sucrose intake was not significantly affected by naloxone pretreatment over the first 15-min of ingestion, but was then

significantly reduced over the remaining 45 min of the 1-h test. In the present study, central administration of naltrexone produced significant dose-dependent reductions in sucrose intake which took 15-20 min before it reached its peak effects. These data appear to support the hypothesis suggesting opioid mediation of the maintenance, rather than the initiation of intake.

The kappa receptor has been proposed as the major mechanism for the opioid mediation of palatability (Cooper et al., 1985). Whereas the kappa agonists, U50,488H, tifluadom, bremazocine, and ethylketocyclazocine, each stimulate palatable food consumption, the mu agonist, morphine, failed to exert effects (Cooper, 1981; Jackson and Cooper, 1985). Arjune and co-workers (Arjune et al., 1990) have previously shown that Nor-BNI was as effective as naltrexone in inhibiting the hyperphagia following exposure to a palatable high-fat diet. In the present study, Nor-BNI displayed an identical dose-dependent pattern of effects in inhibiting sucrose intake as naltrexone, including the 15-20 min interval needed to exert maximal effects. Previous studies have indicated that Nor-BNI is quite effective in reducing nocturnal food intake (Arjune and Bodnar, 1990), 2DG hyperphagia (Arjune and Bodnar, 1990; Arjune et al., 1990), and high-fat feeding (Islam and Bodnar, 1990; Arjune and Bodnar, 1990), as well as feeding induced by the opioid agonist actions of U50,488H, DAMGO, DSLET, B-FNA and DALCE (Levine et al., 1991). In contrast, Nor-BNI transiently reduced the increased food intake induced by either food deprivation (Arjune et al., 1991; Levine et al., 1990) or insulin

(Beczkowska and Bodnar, 1991). This suggests that the kappa receptor is working quite specifically upon certain types of intake situations, but not others. Whereas potent inhibition occurs for palatable high-fat and sucrose intake, marginal inhibition occurs for food or water intake following deprivation. These data support a specific kappa-mediated modulation of palatability if sucrose is acting purely through orosensory mechanisms.

It should be noted that, similar to deprivation-induced water intake, the Nor-BNI's effects were highly correlated with the animal's baseline sucrose intake. Whereas Nor-BNI was more effective in animals with high baseline water-deprivation intake and less effective in animals with low water-deprivation intake, Nor-BNI was more effective in animals with low baseline sucrose intake and less effective in animals with high baseline sucrose intake. In contrast to presumed primary mu_2 and secondary kappa activation following deprivation-induced water intake, sucrose intake appears very strongly dependent upon primary kappa receptor activation, and secondarily on other opioid receptor subtypes. On the other hand, the inhibitory effects of naltrexone and B-FNA were independent of the amount of sucrose solution an animal consumed under the baseline condition.

The selective mu antagonist, B-FNA, significantly inhibited sucrose intake, but the selective mu_1 antagonist, naloxonazine, failed to significantly alter sucrose intake. The ability of B-FNA ($mu = mu_1 + mu_2$) and the inability of naloxonazine (mu_1) to antagonize sucrose intake indicate a role for the mu_2 receptor subtype in these

effects. This pattern of effects has been observed previously for hyperphagia following 2DG, insulin and exposure to a high-fat diet (e.g. Arjune et al., 1990a; Islam and Bodnar, 1990). In contrast, both B-FNA and naloxonazine were previously effective in reducing spontaneous and deprivation-induced food intake (Arjune et al., 1990b; Simone et al., 1985; Mann et al., 1988). Whereas Nor-BNI was as effective in inhibiting sucrose intake as naltrexone, B-FNA produced smaller magnitudes of inhibition as compared to naltrexone, suggesting a primary role of the kappa receptor in opioid modulation of sucrose intake.

DALCE and naltrindole respectively block $delta_1$ and $delta_2$ receptors, but neither appear to participate actively in the mediation of sucrose intake. Despite the ability of delta-selective agonists to stimulate food intake (e.g. Gosnell et al., 1986) and intake of sweet solutions (Gosnell and Majchrzak, 1989), the delta-specific antagonists have previously failed to alter spontaneous, deprivation and glucoprivic intake as well as intake stimulated by a high-fat diet (Arjune et al., 1991; Beczkowska and Bodnar, 1991). In the present study, naltrindole produced significant inhibition of sucrose intake when compared to saline vehicle. However, this compound is highly unstable and can stay in an aqueous solution only when a specific buffer is used. Administration of the cyclodextrin buffer alone significantly inhibited sucrose intake, and hence alterations in sucrose intake following naltrindole were compared to these buffer effects and failed to reach significance. Thus, as suggested previously (Arjune et al., 1991), delta receptor agonists may be acting

through modulatory, rather than direct actions upon ingestion. Indeed, low doses of the delta antagonists, DALCE and naltrindole, as well as the mu_1 antagonist, naloxonazine, actually stimulated sucrose intake. The mechanisms of action of these effects are not clear. The failure of naloxonazine, DALCE and naltrindole to alter sucrose intake could not be explained by any relationship between the antagonists' potency and basal intake.

In conclusion, there seems to be a differential involvement of opioid receptor subtypes in deprivation-induced water intake and sucrose intake. Deprivation-induced water intake was inhibited by naltrexone and B-FNA, but not by other opioid antagonists suggesting that this form of intake is modulated by the mu_2 opioid receptor. Sucrose intake, on the other hand, was inhibited by naltrexone, B-FNA and Nor-BNI. If the inhibitory effects of opioid antagonists were due exclusively to their antidipsogenic effects, then B-FNA would be as effective in inhibiting sucrose intake as it was effective in inhibiting water intake and there would have been no involvement of kappa receptor antagonist, Nor-BNI. Since B-FNA was less effective than naltrexone, and Nor-BNI was highly effective in inhibiting sucrose intake, it suggests that the effects observed following B-FNA may be secondary to modulation of fluid intake per se, and that the effects observed following Nor-BNI are primarily due to kappa modulation of palatable sucrose intake.

CHAPTER 5. Opioid Antagonists and Saccharin Intake.

Introduction

The antidipsogenic effects of naloxone appear to be more potent in inhibiting sweet fluid intake than water intake (Levine et al., 1982) suggesting that naloxone's inhibition of sucrose intake is most probably due to its attenuation of palatability and not postingestive factors (Kirkham and Cooper, 1988a,b). These results however do not exclude the possibility that there could be a differential involvement of specific opioid receptor subtypes in the modulation of fluid intake that has palatable and nutritive characteristics as compared with intake that is palatable but non-nutritive. To separate nutritional factors from palatability factors, previous experiments evaluated saccharin which has a sweet taste but is non-nutritive.

Opioid involvement in the modulation of saccharin intake was demonstrated by naloxone's (1 mg/kg) marked attenuation of the consumption of a 0.05% saccharin solution in a two-bottle preference test (LeMagnen et al., 1980). Water intake was simultaneously elevated, so that the naloxone-treated animals no longer expressed the preference for the sweet fluid. In contrast, intake of sweet solutions are increased following administration of morphine, the mu-selective opioid agonist, DAMGO, and the delta-selective opioid agonist [D-Thr2]-enkephalin-Thr6 (DTLET) (Calcagnetti and Reid, 1983; Czirr and Reid, 1986; Gosnell and Majchrzak, 1989; Lynch and Libby, 1983; Siviy et al., 1982).

If the reductions in sucrose intake observed in Chapter 4 were exclusively due

to opioid antagonists' ability to alter the palatable/hedonic properties of sucrose, then a similar pattern of highly effective kappa modulation and a significant but small mu_2 modulation would be expected in modulation of saccharin intake. If a different pattern emerges, then it might suggest that the opioid system, in addition to modulating hedonic properties of an ingestate, might also be involved in processes related to postingestive consequences of consumed foods, and thereby play a role in some kind of feedback mechanism involved in either meal maintenance or its termination.

Methods

Protocol: A 0.1% saccharin solution (1 g saccharin/ 1 l water) was prepared fresh before testing with tap water and sodium saccharin crystals (Sigma Chemical Company). To introduce the 0.1% saccharin solution, rats were water-deprived for 24 h, and given access to the 0.1% saccharin solution (55 ml, 1 ml gradations) in a sipper tube attached to the front of the cage for 1 h. Measurement of baseline intakes for saccharin proceeded as described in Chapter 4, and a criterion intake of 10 ml was established in order for the rat to continue in the paradigm.

Each rat received up to 9 microinjection conditions according to a counterbalanced order at weekly intervals at 2-7 h into the light cycle. A first group of rats received: a) vehicle (n=18), b) naltrexone at doses of 1 (n=6), 5 (n=6), 20 (n=8), and 50 (n=6) ug, c) B-FNA at doses of 1 (n=10), 5 (n=10) and 20 (n=14) ug, and d) Nor-BNI at doses 1 (n=10), 5 (n=10) and 20 (n=0) ug. A second group

of rats received: a) vehicle (n=16), b) DALCE at doses of 10 (n=9), 20 (n=9) and 40 (n=9) ug, c) naltrindole at doses of 1 (n=8), 5 (n=8) and 20 (n=8) ug, and buffer (n=8), and d) naloxonazine at doses of 10 (n=7), 20 (n=6), and 50 (n=6) ug. Saccharin intake was assessed at 5-min intervals for 1 h and cumulative intake was recorded. To determine whether each rat recovered its baseline saccharin intake, an injection-free saccharin drinking session occurred 48-72 h after each drug condition. All rats in this paradigm had to recover their baseline intakes to continue in the study.

Results

Naltrexone significantly and dose-dependently inhibited saccharin intake at the 25-60 min intervals of the 1-h time course (Figure 13). Relative to vehicle, only naltrexone doses of 20 (50%) and 50 (61%) ug inhibited saccharin intake after 25 min with the peak effect of the highest dose (50-66%) occurring after 25-60 min of exposure. Decreases in overall saccharin intake occurred following naltrexone doses of 1 (35%), 5 (47%), 20 (55%), and 50 (66%) ug. Naltrexone's inhibition of saccharin intake was not significantly related ($r(8)=-0.002$) to baseline intake.

Nor-BNI failed to significantly inhibit saccharin intake at any of the intake intervals (Figure 14). Nor-BNI (1 and 20 ug) non-significantly inhibited saccharin intake (18-28% and 30-36% respectively) after 5-60 min of exposure with the effects increasing with time. Nor-BNI (5 ug) non-significantly increased intake (8-41%) with the effects decreasing with time. Nor-BNI's failure to alter saccharin intake could not be explained by differences in baseline intake across animals ($r(9)=0.23$).

FIGURE 13. Alterations in intake of a 0.1% saccharin solution (ml) following central microinjections of vehicle (filled circle) and the general opioid antagonist, naltrexone (1 ug - open circle, 5 ug - filled square, 20 ug - open square, 50 ug - filled triangle). Two highest doses of naltrexone significantly inhibited saccharin intake (20 ug: 50%, 25-60 min; 50 ug: 61%, 25-60 min). Decreases in the overall saccharin intake occurred following naltrexone doses of 1 (35%), 5(47%), 20 (55%), and 50 (66%) ug. Standard error range 0.28-3.00.

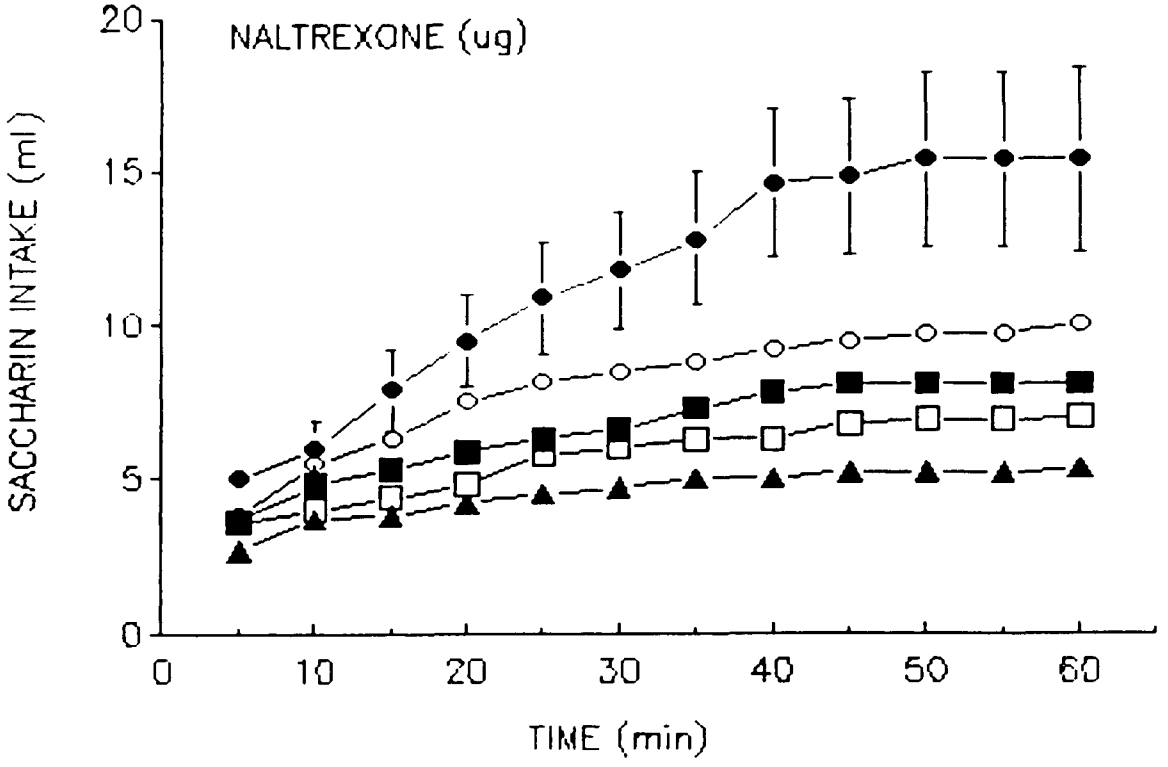
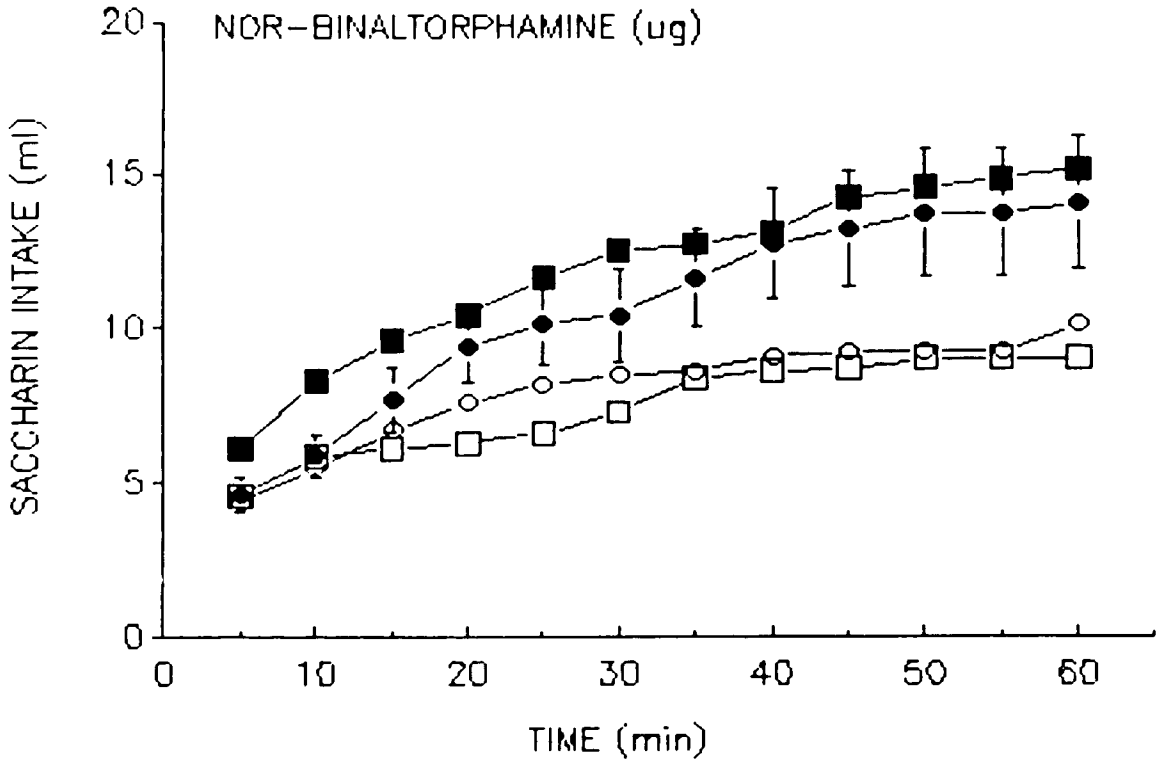

NALTREXONE (ug)
SACCHARIN INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 14. Alterations in intake of a 0.1% saccharin solution (ml) following central microinjections of vehicle (filled circle) and the kappa-selective opioid antagonist, Nor-BNI (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of Nor-BNI non-significantly altered saccharin intake: 1 (28% decrease) and 20 ug (36% decrease), and 5 ug (8% increase). Standard error range 0.57-2.14.


NOR-BINALTORPHAMINE (ug)
SACCHARIN INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

B-FNA failed to significantly inhibit saccharin intake at any of the intake intervals (Figure 15). The two higher doses of B-FNA (5 and 20 ug) non-significantly increased saccharin intake (8-40%) after 15-20 min of exposure. B-FNA's failure to alter saccharin intake could not be explained by differences in baseline intake across animals ($r(12)=0.31$).

Since the buffer solution significantly increased saccharin intake (30%-40%) relative to a distilled water vehicle, the effects of naltrindole were evaluated relative to this buffer. Naltrindole (20 ug) significantly inhibited saccharin intake (75-94%) at each of the 5-min intervals over the 1-h time course (Figure 16). Naltrindole (1 and 5 ug) non-significantly decreased saccharin intake (12-24% and 15-23% respectively) over the entire time course with the effects strongest at the onset. Naltrindole's inhibition of saccharin intake was not significantly related ($r(7)=0.15$) to baseline intake.

DALCE (40 ug) significantly inhibited saccharin intake (61%) in the first 5 min of exposure (Figure 17). DALCE (10 and 20 ug) non-significantly inhibited saccharin intake (18% and 29% respectively) over the entire time course. DALCE's inhibition of saccharin intake was not significantly related ($r(9)=-0.61$) to the baseline intake.

Naloxonazine failed to significantly alter saccharin intake at any of the 5-min intervals over the 1-h time course (Figure 18). Relative to vehicle, naloxonazine (10, 20 and 50 ug) non-significantly stimulated saccharin intake at doses of 10 (3-16%),

FIGURE 15. Alterations in intake of a 0.1% saccharin solution (ml) following central microinjections of vehicle (filled circle) and the mu selective opioid antagonist, B-FNA (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of B-FNA non-significantly decreased saccharin intake: 1 (17%), 5 (1%) and 20 (4%) ug. Standard error range 0.70-1.00.

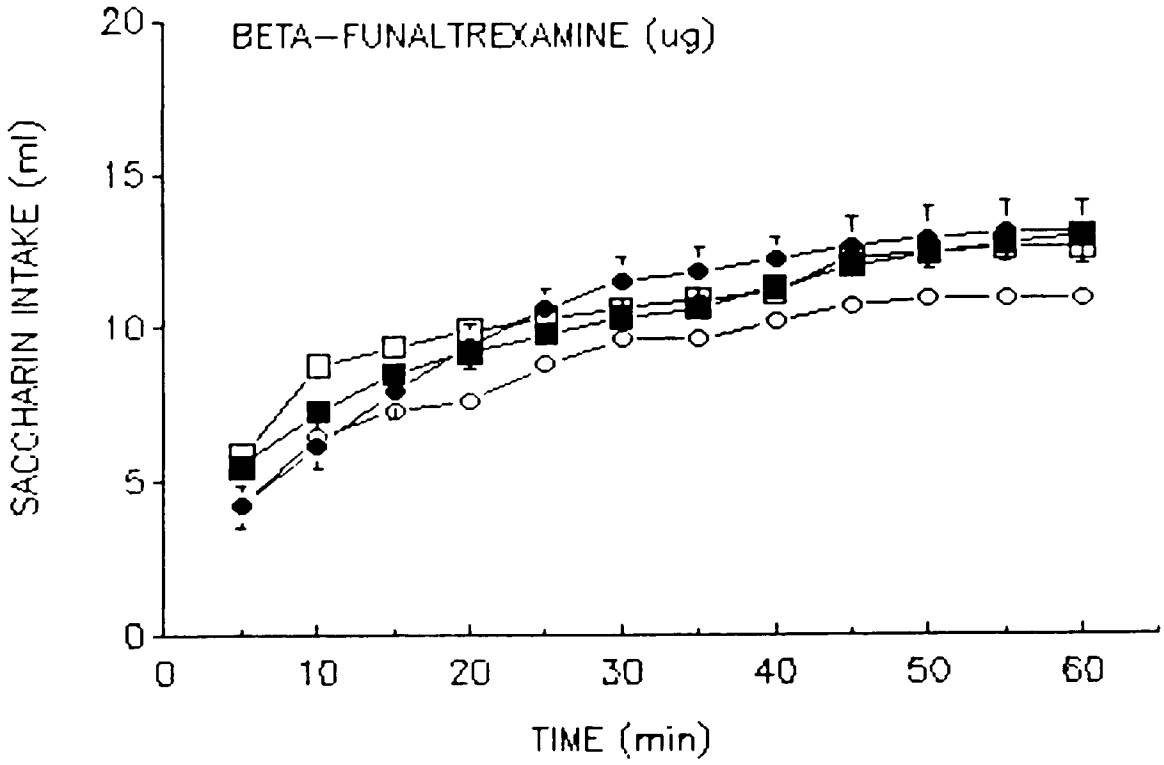

BETA–FUNALTREXAMINE (ug)
SACCHARIN INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 16. Alterations in intake of a 0.1% saccharin solution (ml) following central microinjections of vehicle buffer (filled circle) and the delta$_2$-selective opioid antagonist, naltrindole (1 ug - open circle, 5 ug - filled square, 20 ug - open square). Whereas the 20 ug dose of naltrindole significantly inhibited saccharin intake (75%, 5-60 min), saccharin intake was non-significantly reduced following naltrindole doses of 1 (24%) and 5 (23%) ug. Standard error range 0.91-1.54.

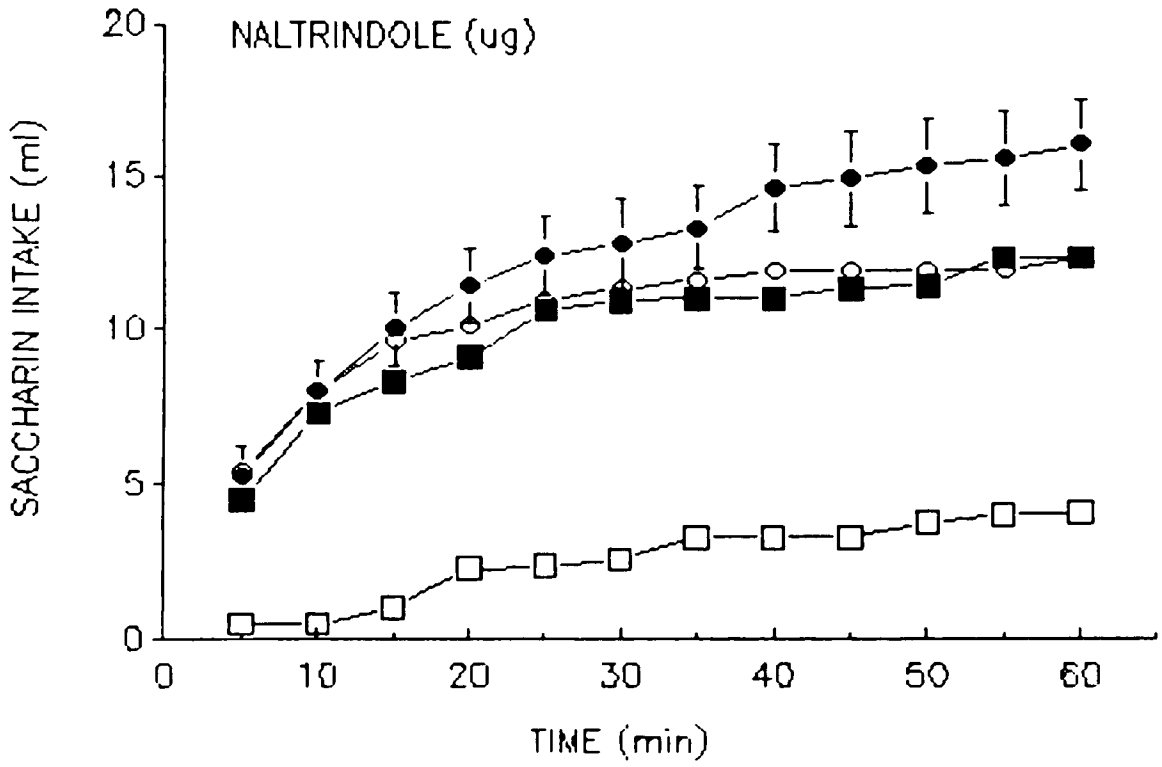

NALTRINDOLE (ug)
SACCHARIN INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 17. Alterations in intake of a 0.1% saccharin solution (ml) following central microinjections of vehicle (filled circle) and the $delta_1$-selective opioid antagonist, DALCE (10 ug - open circle, 20 ug - filled square, 40 ug - open square). Whereas the 40 ug dose of DALCE significantly inhibited saccharin intake (61%, 5 min), DALCE non-significantly reduced saccharin intake at doses of 10 (18%), 20 (29%) and 40 (41%) ug. Standard error range 0.68-1.30.

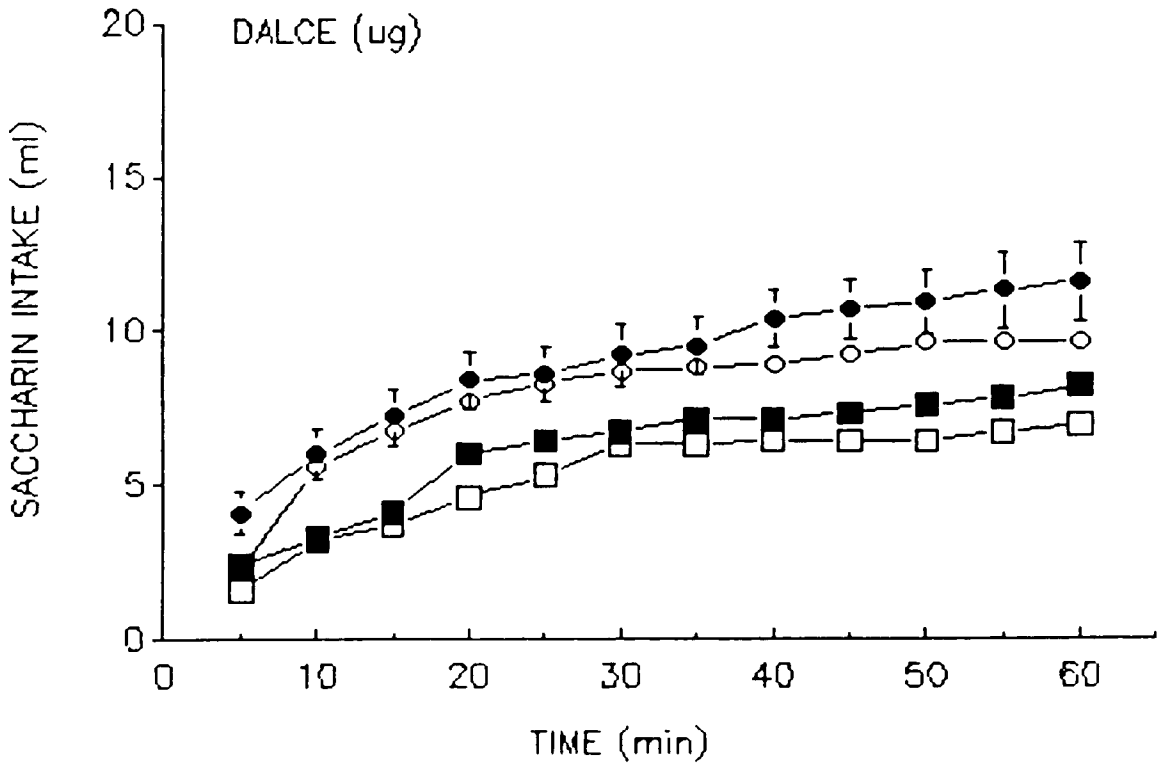
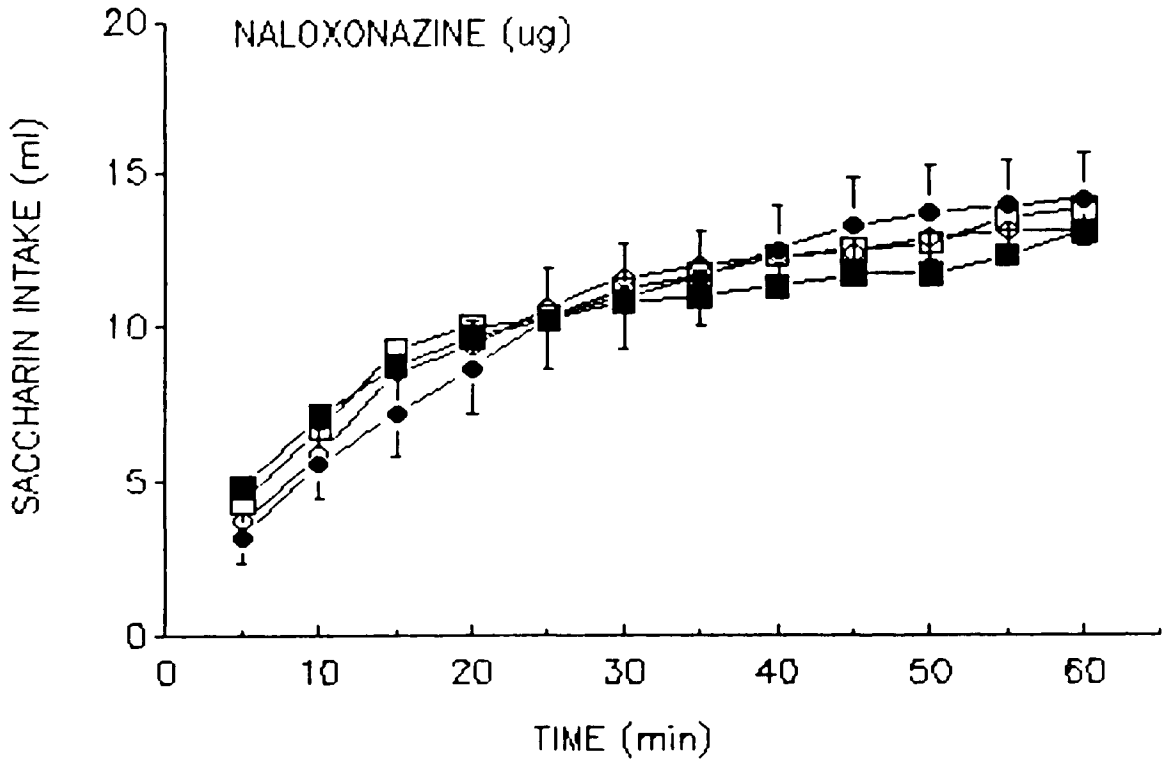

DALCE (ug)
SACCHARIN INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 18. Alterations in the intake of a 0.1% saccharin solution (ml) following central microinjections of vehicle (filled circle) and the mu_1-selective opioid antagonist, naloxonazine (10 ug - open circle, 20 ug - filled square, 50 ug - open square). All three doses of naloxonazine non-significantly reduced saccharin intake following 10 (8%), 20 (8%) and 50 (2%) ug doses. Standard error range 0.81-1.69.


NALOXONAZINE (ug)
SACCHARIN INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

20 (11-50%) and 50 (1-34%) ug after 5-20 min. Naloxonazine non-significantly inhibited intake (8%) after 25-60 min of exposure. Naloxonazine's failure to inhibit saccharin intake could not be explained by differences in baseline intakes across animals ($r(7) = -0.53$).

Discussion

Central administration of naltrexone significantly inhibited saccharin intake, however, it was effective only at the two highest doses. Only the highest dose of the $delta_2$ antagonist, naltrindole, significantly inhibited saccharin intake throughout the entire time course with a greater magnitude than naltrexone. The $delta_1$ antagonist, DALCE, inhibited saccharin intake only at 5 min of exposure. The mu antagonist, B-FNA, and the mu_1 antagonist, naloxonazine, transiently stimulated saccharin intake, whereas the kappa antagonist, Nor-BNI, non-significantly reduced saccharin intake over the entire time course. The failure of the above- mentioned opioid antagonists could not be explained by differences in basal intakes since none of the effects observed with saccharin were significantly correlated to its basal intake.

Although the 25-min delay in the development of naltrexone's inhibitory effects suggests a role for postingestive factors, saccharin's lack of nutritive value eliminates this explanation. Kirkham and Blundell (1984, 1986) have proposed that general opiate antagonists interfere with the maintenance, rather than the initiation, of intake. Whereas peripheral naloxone reduces sucrose intake in sham-feeding animals, it only becomes effective after 15 min of exposure (Kirkham and Cooper,

1988a,b). Both the sucrose and saccharin data appear to support the hypothesis suggesting general opioid mediation of the maintenance, rather than the initiation of intake. These results seem to be in agreement with a previously- suggested role of the endogenous opioid system in the mediation of palatability (Cooper et al., 1985; Morley and Levine, 1983).

The $delta_2$ antagonist, naltrindole, significantly inhibited saccharin intake with the greatest magnitude of effect at the onset of the drinking session. In contrast, DALCE, the $delta_1$ receptor antagonist, significantly reduced saccharin intake, but only in the first 5 min of the session. However, these results should be considered in light of naltrindole's limited ability to inhibit saccharin intake only at a 20 ug dose, but not at doses of 1 or 5 ug. Further, in addition to DALCE's temporally-limited effects at 40 ug, this antagonist failed to alter saccharin intake at any of the lower doses tested. Despite these limitations, these data are in agreement with the findings (Gosnell and Majchrzak, 1989) that delta-selective agonists stimulate intake of sweet solutions, and suggest that the $delta_1$ opioid receptor subtype is involved in the opioid modulation of saccharin intake. The present effects are in contrast to this dissertation's findings that neither delta antagonist significantly altered deprivation-induced water intake or sucrose intake. In addition, it should be noted that inhibition of saccharin intake by naltrindole and DALCE is one of the very few ingestive situations in which delta antagonists exert inhibitory effects. The reversible delta receptor antagonist, ICI174864, transiently decreased nocturnal feeding

(Jackson and Sewell, 1985) and high-fat intake (Islam and Bodnar, 1990), but only at doses that produce motor dysfunction (Long et al., 1988). In contrast, the $delta_1$ antagonist, DALCE failed to alter free feeding, and hyperphagia following food deprivation, 2DG, insulin and high-fat exposure (Arjune et al., 1991; Beczkowska and Bodnar, 1991). Naltrindole also failed to alter deprivation-induced food intake (AS Levine, personal communication).

B-FNA significantly inhibited deprivation-induced water intake and sucrose intake. Nor-BNI was highly effective in significantly inhibiting sucrose intake at very low doses. Therefore, it was quite surprising that neither of these antagonists were effective in significantly reducing saccharin intake. Since these failures could not be explained by any dichotomies in the relationship between these antagonists' effects and baseline saccharin intakes, a preliminary study was conducted to explore whether simultaneous blockades of both mu and kappa receptors and of both $delta_2$ and kappa receptors were necessary to produce inhibitory effects upon saccharin intake. To this end, a separate group of rats was assessed for saccharin intake following pairs of vehicle injections, following pretreatment with B-FNA (20 ug, 24 h) and Nor-BNI (20 ug, 0.5 h), and following pretreatment with DALCE (40 ug, 24 h) and Nor-BNI (20 ug, 0.5 h). Neither of the antagonist combinations significantly affected saccharin intake, suggesting that neither mu blockade alone, kappa blockade alone, mu and kappa blockade, nor $delta_1$ and kappa blockade is sufficient to inhibit saccharin intake. Other possible factors that should be considered in the differential effects

of opioid receptor subtype antagonists upon sucrose and saccharin intake are the percentage of animals that met the required criterion for each form of intake, and total intake for each form of intake. The criteria in these studies were such that all animals must sample the ingestate within 5 min, and consume a minimum of 10 ml over 1 h following vehicle treatment. In the sucrose paradigm, virtually all (>90%) of the animals met the criterion, and proceeded into the antagonist paradigm. In contrast, fewer (75%) of animals tested for saccharin consumption met the necessary criterion. Further, in examining the overall 1 h consumption following vehicle treatment, sucrose intake (17.1 ml) was higher than saccharin intake (13.4 ml). These data suggest that sucrose may be more preferred than saccharin, possibly because it has both palatable and post-ingestive consequences.

Three possible mechanisms have been advanced to explain opioid antagonist effects upon intake of palatable solutions. The first mechanism proposes that opioid antagonists are exerting their inhibitory effects upon palatability as part of their overall inhibition of fluid intake per se. This was addressed in Chapter 3, and these data indicated that deprivation-induced water intake was inhibited by general and mu antagonists, but not by kappa, mu_1, $delta_1$ and $delta_2$ antagonists. The pattern of effects induced by deprivation-induced water intake does not match that observed for saccharin intake, and does not appear to explain the latter results. The second mechanism proposes that opioid antagonists are exerting their inhibitory effects upon palatability together with post-ingestive consequences. This was addressed in

Chapter 4, and these data indicated that sucrose intake was inhibited by general, kappa and mu antagonists, but not by mu_1, $delta_1$ and $delta_2$ antagonists. Again the pattern of effects observed with sucrose intake does not match that observed for saccharin intake, and does not appear to explain the latter results.

The third mechanism proposes that opioid antagonists are exerting their inhibitory effects upon the hedonic qualities of the ingestate alone, that is, palatability factors are paramount in the consequences of the effects. This mechanism is well-suited to the inherent properties of saccharin in rats. Although saccharin solutions have been reported to have both sweet and bitter tastes in humans (Morrison & Jessup, 1977), rats fail to generalize a conditioned saccharin aversion to other bitter stimuli (Stewart and Krafczek, 1988). These data suggest that saccharin at low concentrations does not taste bitter to rats, and that it is the hedonic sweet taste of saccharin that is modulating its intake. Indeed, a taste receptor specific for saccharin has been proposed in electrophysiological studies (Jakinovich and Sugarman, 1988). Thus, the relative effectiveness of general and delta receptor antagonists and the ineffectiveness of kappa and mu antagonists in inhibiting saccharin intake may reflect saccharin's ability to stimulate intake through palatability mechanisms with no post-ingestive consequences.

Since Nor-BNI and B-FNA were found to be ineffective in altering saccharin intake, yet effective in altering sucrose intake, it would appear that the nutritive value of sucrose played a major role in the primary activation of kappa receptors and

secondary activation of mu receptors. It is conceivable that the ad libitum ingestion of sucrose is part of a dietary self-selection strategy that is dependent upon post-ingestive factors, and that the opioid modulation of sucrose intake might reflect alterations in macronutrient self-selection. Initial studies indicated that general opioid agonists selectively stimulated fat consumption (e.g.: Marks-Kaufman, 1982; Marks-Kaufman and Kanarek, 1980; Marks-Kaufman and Lipeles, 1982; Rosmos et al., 1987), and that general opioid antagonists selectively inhibited fat consumption (Marks-Kaufman and Kanarek, 1981, 1990; Marks-Kaufman et al., 1985). More recent studies suggest a more complex relationship between opioid function and macronutrient selection. In this regard, morphine increased protein intake in protein-deprived rats, and slightly increased carbohydrate intake in carbohydrate-deprived rats (Evans and Vaccarino, 1990). Further, the increased intake by morphine in rats characterized as either fat-preferring or carbohydrate-preferring correlated with their respective preferred macronutrient (Gosnell et al., 1990). Leibowitz and co-workers found that morphine selectively increased protein intake in non-deprived rats in one study (Shor-Posner et al., 1986), but increased intake of all three macronutrients in non-deprived rats in a second study (Bhakthavatsalam and Leibowitz, 1986). The increases in fat and protein intakes in the latter study were greater than that of carbohydrate intake.

In conclusion, the differential pattern of results observed with saccharin (general and delta antagonists) as compared to sucrose (general, kappa and mu

antagonists) suggests that the lack of post-ingestive consequences plays an important role in determining which subset of opioid receptors are activated by saccharin. A potential limitation that could explain the differences in baseline intake between sucrose and saccharin is that perhaps it is possible that if lower concentration of sucrose was employed the intake would have been lower. Further studies need to explore this question. Nevertheless, since sucrose has post-ingestive consequences, it would appear that the previously-postulated role of the kappa receptor in palatability (Cooper et al., 1985; Morley and Levine, 1983) must be limited to those palatable situations that have post-ingestive effects (e.g., sucrose and fat). Indeed, the highly-potent effects of Nor-BNI upon sucrose intake, together with its inability to alter saccharin intake suggests that the kappa receptor may be modulating short-term post-ingestive effects. Post-ingestive consequences are a function of which macronutrient (fat, protein, carbohydrate) has been selected and consumed. Nor-BNI significantly and potently inhibited high-fat (Arjune and Bodnar, 1990) and sucrose intake, indicating its modulation of fat and carbohydrate macronutrients respectively. B-FNA exerted a similar pattern of effects (Islam and Bodnar, 1990; Chapter 4), but with lesser potency and magnitude. The following chapter examined the role of general and specific opioid receptors in carbohydrate intake further by assessing general and selective opioid antagonist effects upon complex carbohydrate intake.

CHAPTER 6. Opioid Antagonists and CMD Intake.

Introduction

The discovery of the rat's polysaccharide taste sensitivity emerged from experiments conducted with Polycose, a corn starch hydrolysate. Rats readily consume Polycose solutions and, at low concentrations, prefer Polycose to sucrose, glucose, fructose, and maltose solutions (Sclafani and Clyne, 1987; Sclafani and Mann, 1987). Furthermore, the preference threshold for Polycose is 26 times lower than that for sucrose and maltose (Sclafani and Nissenbaum, 1987). Based on this evidence, Sclafani (1987) proposed that rats have two different taste systems for carbohydrates: a sweet taste system for the detection of sugar-rich foods, and the polysaccharide taste system which presumably is not stimulated by intact water-insoluble starch molecules. This latter system is activated by smaller, soluble polysaccharide molecules cleaved from starch by the action of salivary amylase.

Although it is reasonable to expect that the opioid system would be involved in modulation of yet another palatable substance, it is not clear whether its involvement is limited to simple sugars or whether it extends to complex carbohydrates as well. Although morphine increased the intake of protein-rich diet in protein-deprived rats, it only slightly increased intake of a carbohydrate-rich diet in carbohydrate-deprived rats (Evans and Vaccarino, 1990). In contrast, morphine increased intake of the preferred macronutrient in fat-preferring and carbohydrate-preferring rats (Gosnell et al., 1990). Bhakthavatsalam and Leibowitz (1986)

reported that in non-deprived rats, morphine increased the intake of all three macronutrients, although the increases in fat and protein intake were greater than that of carbohydrate intake. Based on these studies it seems that the opioid system should be relatively ineffective in modulating carbohydrate intake. Yet Chapter 4 illustrated a strong kappa opioid receptor involvement in the modulation of intake of a 10% sucrose solution which belongs to a carbohydrate food group. Therefore, the modulation of carbohydrate intake by endogenous opioids may be related to the intensity of the macronutrient in the ingestate, and can be tested by allowing animals access to a concentrated solution. Since concentrated solutions of both sucrose and complex carbohydrates have hedonic properties and postingestive consequences, it is of interest to determine whether general and specific opioid receptor modulation of simple carbohydrates extends to complex carbohydrates as well. To test this hypothesis, the polysaccharide carbohydrate maltose dextrin (CMD) was tested to answer the following questions: 1) Is the involvement of the opioid system specific to a sweet taste or the palatability factor in general? 2) Is the involvement of the opioid system in modulation of the carbohydrate intake specific to the simple sugars or does it extend to polysaccharides? 3) Is the opioid system involved only in palatability or is it also involved in feedback mechanism related to postingestive consequences of consumed foods that maintain/terminate the meal?

Methods

Protocol: A 10% CMD solution (100 g CMD/ 1 l water) was prepared fresh

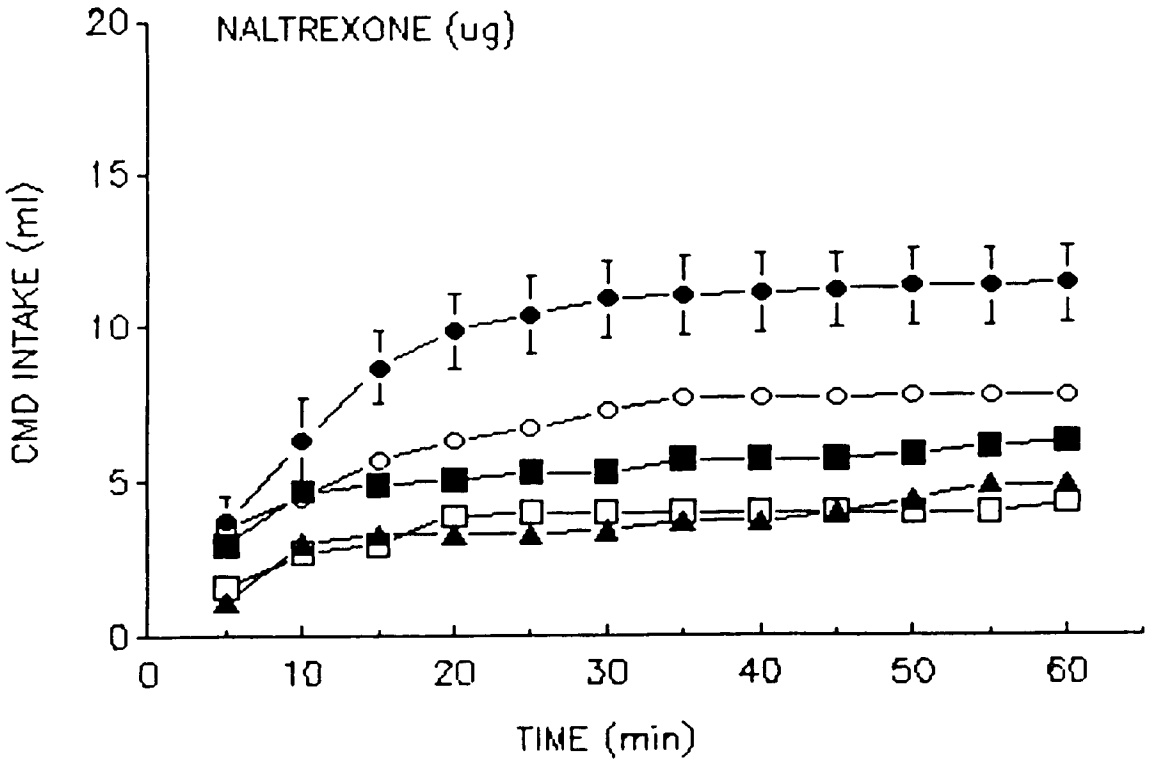
before testing with tap water and carbohydrate maltose dextrin (BioServ Incorporated). To introduce the 10% CMD solution, rats were water-deprived for 24 h, and given access to the CMD solution (55 ml, 1 ml gradations) in a sipper tube attached to the front of the cage for 1 h. Measurement of baseline intakes for CMD proceeded as described in Chapter 4, and a criterion intake of 10 ml was established in order for the rat to continue in the paradigm.

Each rat received up to 9 microinjection conditions according to a counterbalanced order at weekly intervals at 2-7 h into the light cycle. A first group of rats received: a) vehicle (n=12), b) naltrexone at doses of 1 (n=6), 5 (n=7), 20 (n=7), and 50 (n=7) ug, c) B-FNA at doses of 1 (n=7), 5 (n=7) and 20 (n=8) ug, and d) Nor-BNI at doses 1 (n=7), 5 (n=7) and 20 (n=8) ug. A second group of rats received: a) vehicle (n=14), b) DALCE at doses of 10 (n=7), 20 (n=7) and 40 (n=7) ug, c) naltrindole at doses of 1 (n=7), 5 (n=6) and 20 (n=10) ug, and buffer (n=7), and d) naloxonazine at doses of 10 (n=8), 20 (n=8), and 50 (n=9) ug. CMD intake was assessed at 5-min intervals for 1 h and cumulative intake was recorded. To determine whether each rat recovered its baseline CMD intake, an injection-free CMD drinking session occurred 48-72 h after each drug condition. All rats in this paradigm had to recover their baseline intakes to continue in the study.

Results

Naltrexone significantly and dose-dependently inhibited CMD intake over the 1-h time course (Figure 19). Relative to vehicle, the three higher doses of naltrexone

FIGURE 19. Alterations in intake of a 10% CMD solution (ml) following central microinjections of vehicle (filled circle) and the general opioid antagonist, naltrexone (1 ug - open circle, 5 ug - filled square, 20 ug - open square, 50 ug - filled triangle). The three higher doses of naltrexone significantly and dose-dependently inhibited CMD intake (5 ug: 45%, 15-60; 20 ug: 62%, 15-60 min; and 50 ug: 68%) after 15-60 min. A nonsignificant decrease in CMD intake occurred following the 1 ug dose of naltrexone (32%). Standard error range 0.88-1.36.


NALTREXONE (ug)
CMD INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

(5 ug: 44-51%; 20 ug: 61-64%; and 50 ug : 63-69%) significantly inhibited CMD intake after 15-60 min of exposure. Naltrexone (1 ug) non-significantly inhibited intake (33%) over the entire time course. Naltrexone's inhibition of CMD intake was not significantly related ($r(7) = -0.52$) to baseline intake.

Nor-BNI failed to significantly inhibit CMD intake at any of the intake intervals (Figure 20). Relative to vehicle, Nor-BNI (1 ug: 8-32%, and 5 ug: 5-20%) non-significantly increased intake in the first 5-15 min of exposure. Nor-BNI's alterations in CMD intake was significantly related to baseline intake ($r(8) = -0.74$, $p < 0.05$). Nor-BNI reduced CMD intake in rats with high baseline intakes, but failed to reduce intake in rats with low baseline intakes.

B-FNA (5 and 20 ug) significantly inhibited CMD intake by 40-44% after 20-60 min (Figure 21). B-FNA (1 ug) significantly inhibited intake (39%) after 25-60 min with the effects increasing over time. A significant relationship between baseline intake and B-FNA-induced inhibition of CMD was observed ($r(8) = -0.72$, $p < 0.05$) such that B-FNA was most effective in reducing CMD intake in rats with high baseline intakes.

In keeping with the previous studies, the effects of naltrindole were evaluated relative to its buffer vehicle. Naltrindole failed to significantly alter CMD intake at any of the intake intervals (Figure 22). Whereas naltrindole doses of 1 (4-44%) and 5 (28-122%) ug non-significantly increased CMD intake after 15 min of exposure, the lower dose decreased CMD intake by 14% after 20-60 min. Naltrindole (20 ug) non

FIGURE 20. Alterations in intake of a 10% CMD solution (ml) following central microinjections of vehicle (filled circle) and the kappa-selective opioid antagonist, Nor-BNI (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of Nor-BNI non significantly decreased CMD intake: 1 (11%), 5 (17%) and 20 (34%) ug. Standard error range 0.69-1.74.

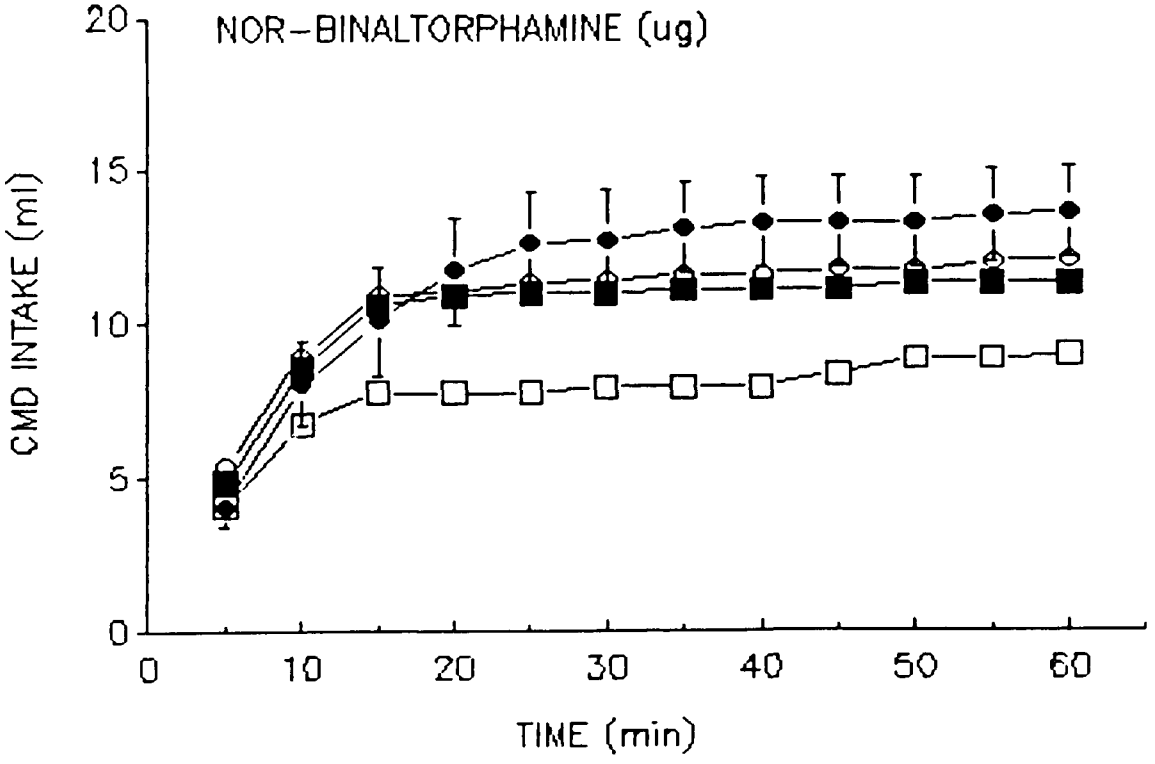

NOR-BINALTORPHAMINE (ug)
CMD INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 21. Alterations in intake of a 10% CMD solution (ml) following central microinjections of vehicle (filled circle) and the mu-selective opioid antagonist, B-FNA (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of B-FNA significantly and dose dependently inhibited CMD intake (1 ug: 39%, 25-60 min; 5 ug: 44%, 20-60 min; 20 ug: 44%, 20-60 min). Standard error range 0.81-1.17.

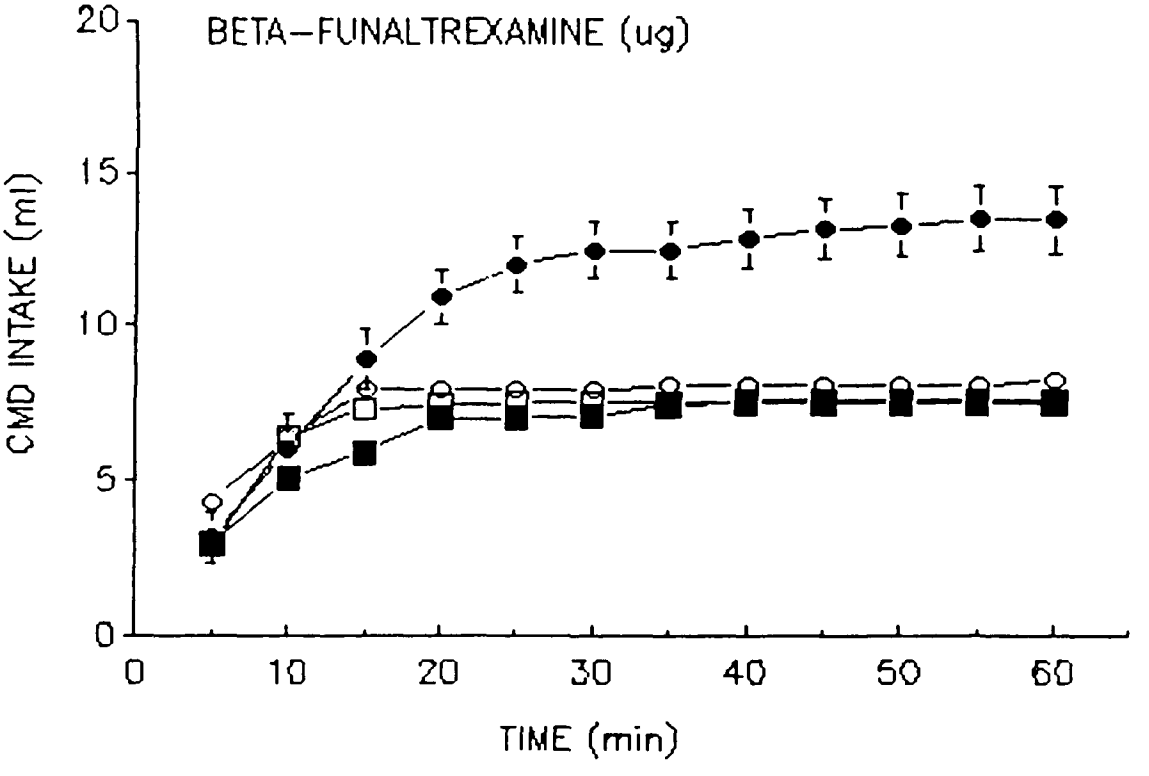
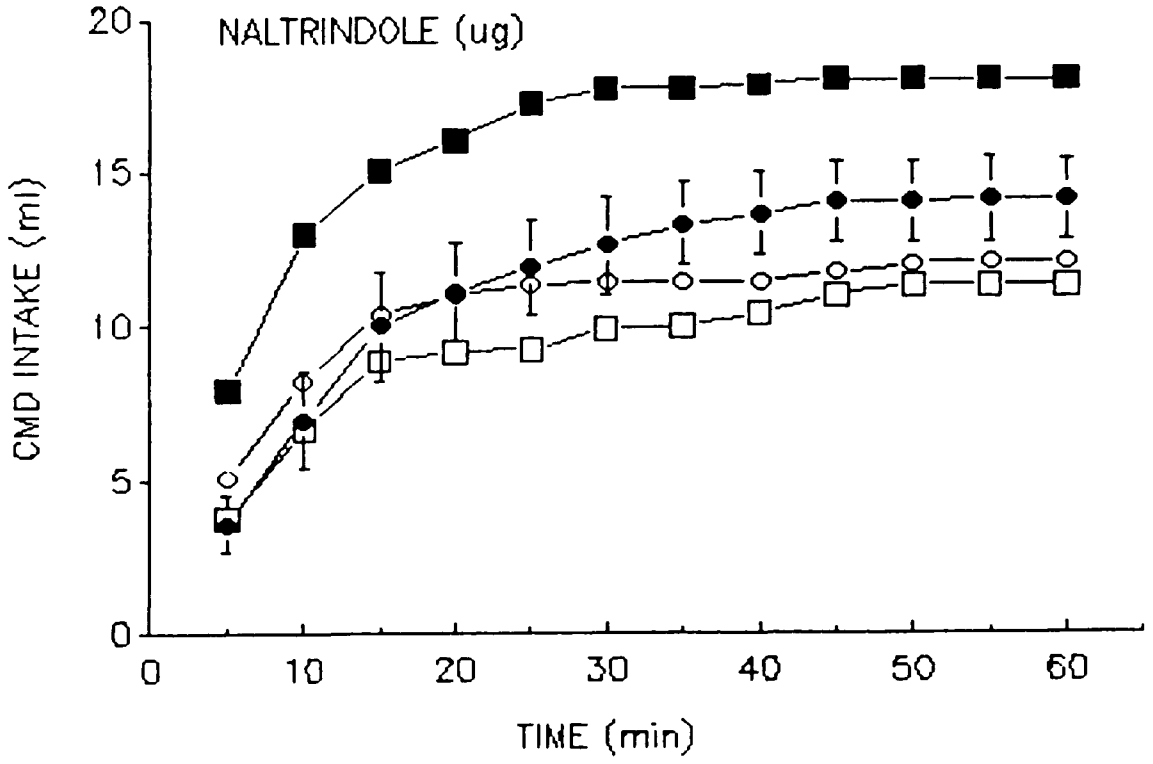

BETA-FUNALTREXAMINE (ug)
CMD INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 22. Alterations in intake of a 10% CMD solution (ml) following central microinjections of buffer vehicle (filled circle) and the $delta_2$-selective opioid antagonist, naltrindole (1 ug - open circle, 5 ug - filled square, 20 ug - open square). All three doses of naltrindole non-significantly altered CMD intake: 1 ug (14% decrease), 5 ug (28% increase) and 20 ug (20% decrease). Standard error range 0.91-1.76.


NALTRINDOLE (ug)
CMD INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

-significantly decreased CMD intake by 25% with the most potent effects after 35 min of exposure. Naltrindole's failure to significantly alter CMD intake could not be explained by any relationship with baseline intake ($r(10) = 0.04$).

DALCE failed to significantly alter CMD intake at any intake intervals (Figure 23). DALCE (10 ug) non-significantly stimulated CMD intake (15-17%) over the time course, whereas DALCE doses of 20 (15%) and 40 ug (2%) non-significantly decreased CMD intake after 5 min of exposure. Although there was a significant inverse relationship between vehicle intake and DALCE-induced effects ($r(7) = -0.90$, $p < 0.05$), evaluation of the individual effects indicated that DALCE never produced appreciable inhibition of CMD intake.

Naloxonazine failed to significantly alter CMD intake at any intake interval (Figure 24). Naloxonazine (10 ug) non-significantly stimulated CMD intake (2-97%) after 5-40 min of exposure. Naloxonazine doses of 20 (5-58%) and 50 (6-90%) ug non-significantly increased CMD intake after 5-15 min, and then inhibited intake (19-26% and 18-22% respectively). Naloxonazine's failure to significantly alter CMD intake was not related ($r(9) = -0.48$) to baseline intake.

Discussion

CMD intake was significantly reduced following the general antagonist, naltrexone, and the mu antagonist, B-FNA, but was unaffected by selective kappa, mu_1, $delta_1$ and $delta_2$ antagonists. Central administration of naltrexone significantly and dose-dependently inhibited CMD intake and the effects took 15 min to develop.

FIGURE 23. Alterations in intake of a 10% CMD solution (ml) following central microinjections of vehicle (filled circle) and the $delta_1$-selective opioid antagonist, DALCE (10 ug - open circle, 20 ug - filled square, 40 ug - open square). All three doses of DALCE non-significantly stimulated CMD intake: 10 ug (17%), 20 ug (24%) and 40 ug (3%). Standard error range 0.81-1.50.

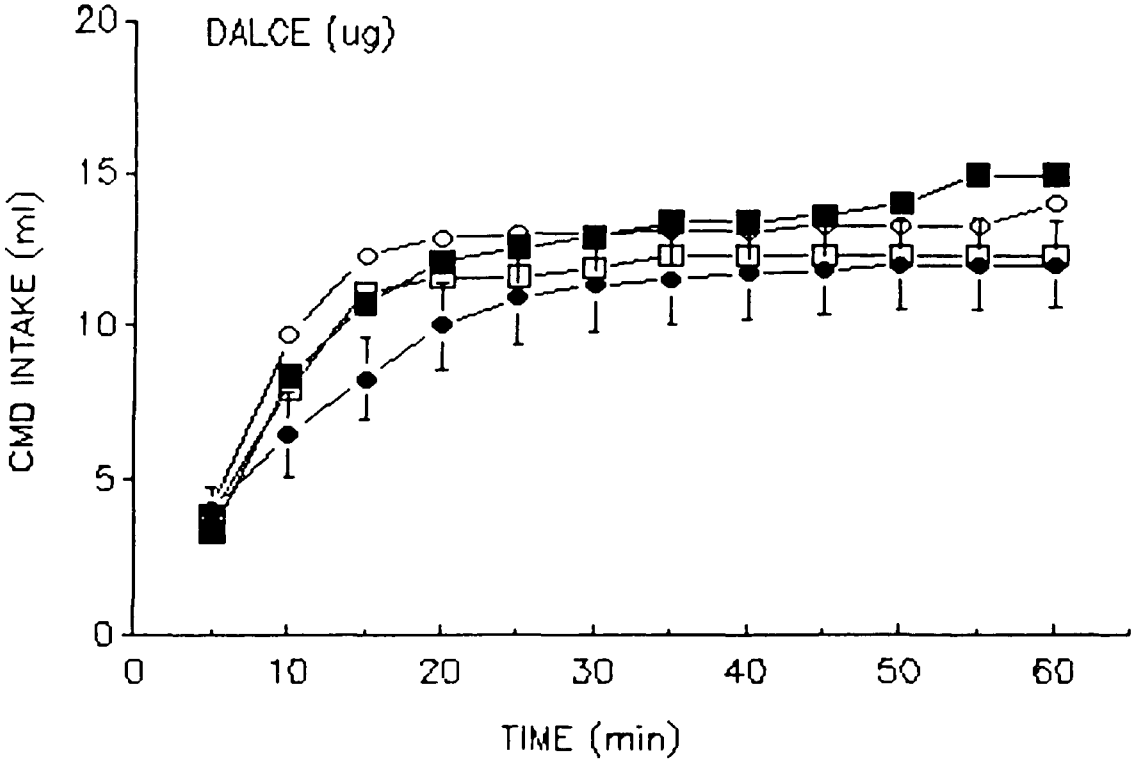
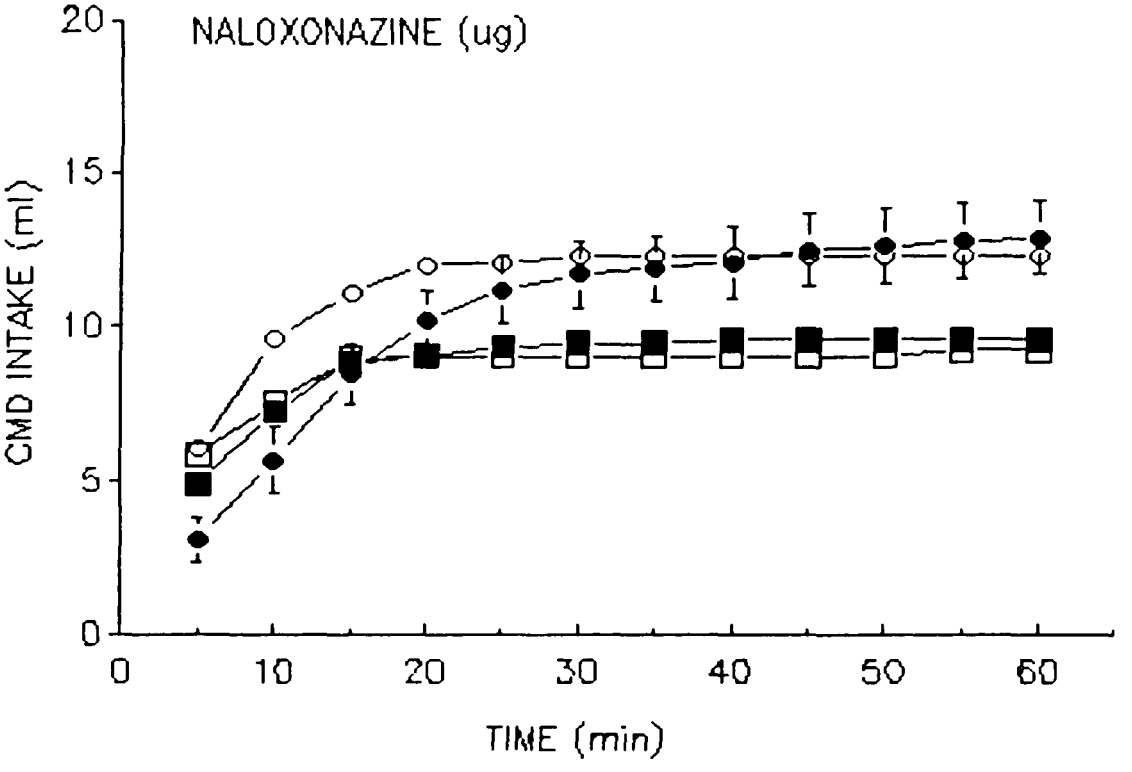

DALCE (ug)
CMD INTAKE (ml)
TIME (min)
0
5
10
15
20
0
10
20
30
40
50
60

FIGURE 24. Alterations in intake of a 10% CMD solution (ml) following central microinjections of vehicle (filled circle) and the mu_1-selective opioid antagonist, naloxonazine (10 ug - open circle, 20 ug - filled square, 50 ug - open square). All three doses of naloxonazine non-significantly reduced CMD intake: 10 ug (5%), 20 ug (26%) and 50 ug (18%). Standard error range 0.73-1.20.


NALOXONAZINE (ug)
CMD INTAKE (ml)
20
15
10
5
0
0
10
20
30
40
50
60
TIME (min)

Although studies with general opioid agonists suggested that involvement of the opioid system in the modulation of carbohydrate intake is minimal, the present study provides evidence that the opioid system alters intake of complex polysaccharide. Combined with the results from Chapter 4, which illustrated that the opioid system is involved in modulation of an intake of a simple sugar, it is reasonable to conclude that the endogenous opioids are involved in modulation of carbohydrates, regardless of their complexity and taste. On the other hand, it should be noted that the animals that participated in these studies were selected according to a criterion of a minimal intake of 10 ml of given solution under non-deprived conditions. Gosnell and coworkers (Gosnell et al., 1990) reported that large individual differences in diet selection allowed the identification of groups of rats characterized as either fat-preferring or carbohydrate-preferring, and this preference was found to correlate with the changes in food intake and diet selection observed after systemic injections of morphine. It is therefore possible that the experimental criteria in essence selected "carbohydrate-preferring" rats and that generalization of these results should be limited to this sub-group of animals.

Central administration of B-FNA, the mu antagonist, significantly and dose-dependently inhibited CMD intake and the effects took 20 to 25 min to develop. On the other hand, naloxonazine, the mu_1 receptor antagonist, non-significantly and transiently stimulated CMD intake. The ability of B-FNA (mu_1 + mu_2) and the inability of naloxonazine (mu_1) to reduce CMD intake suggests the involvement of

the mu_2 opioid receptor in modulating the intake of CMD. In addition, there was a significant relationship between the ability of B-FNA to inhibit CMD intake and the amount of CMD consumed under baseline conditions. B-FNA was most potent in reducing intake in animals with high baseline intake of CMD, and was less effective in reducing intake of animals with low baseline CMD intake. Comparisons of the inhibitory effects of general and mu antagonists revealed a greater magnitude of inhibition for naltrexone (70%) than for B-FNA (45%). These data suggest that opioid modulation of CMD intake is not limited solely to the mu_2 receptor, and that further studies employing multiple blockade of opioid receptor subtypes may reveal which subtype interacts with the mu_2 receptor in mediating CMD intake. In contrast to its inhibition of sucrose intake, Nor-BNI, the kappa-selective antagonist, failed to significantly alter CMD intake. Indeed whereas Nor-BNI inhibition was positively related to baseline sucrose intake, Nor-BNI was more potent in animals with high baseline CMD intake and ineffective in animals with low baseline CMD intake. Whereas it appears that there is a primary regulatory role for the kappa receptor in the modulation of sucrose intake, these results suggest that the involvement of the kappa receptor in the regulation of complex carbohydrate may be secondary to other mechanisms. One possible explanation for these differential results is the existence of two different taste systems for carbohydrates (see reviews: Sclafani, 1987,1991). A sweet taste system is used for the detection of sugar-rich foods, and a polysaccharide taste system is used for the detection of starch-rich foods. In this

model, sugar and polysaccharide taste sensations evoke unconditioned hedonic responses modulated by the energy state. Orosensory stimulation may modify the hedonic evaluation of the particular carbohydrate. The nutritive value of the carbohydrate determines the temporal characteristics of the meal as well as the total intake. This model is in keeping with the postulated opioid role in the maintenance, rather than the initiation of ingestion (Kirkham and Blundell, 1984, 1986; Kirkham and Cooper, 1988a,b). The anatomical, physiological and chemical characteristics of these proposed systems still need to be defined, but the present data would postulate a kappa synapse in the simple sugar taste system, and a mu_2 (and other) synapse in the polysaccharide taste system.

CHAPTER 7. Opioid Antagonist Dose-Response Analysis of effects upon Deprivation-induced, Sucrose, Saccharin and CMD Intake.

To evaluate the relative efficacies of each antagonist in regulating each of the four types of intake, regression analyses were performed on each dose-response function to yield slopes, intercepts and calculation of the ID_{50} in molar concentration (Table 3 which also includes antagonists doses employed in the study converted to molar concentrations for the reference point).

As indicated previously, naltrexone significantly decreased each of the four types of intake, and, as the prototypical opioid antagonist, the potencies of the selective antagonists were compared against it (Table 3). Although naltrexone was quite effective in significantly inhibiting each form of intake by 50% at relatively low doses, it was more potent in reducing CMD intake (ID_{50}=30 mM) and less potent in reducing deprivation-induced water intake (ID_{50}=90 mM).

The kappa antagonist, Nor-BNI only significantly decreased sucrose intake when comparing antagonist effects with vehicle. In this regard, Nor-BNI (ID_{50}=30 mM) was more potent than naltrexone (ID_{50}=50 mM) in inhibiting this form of intake. Despite the failure of Nor-BNI to significantly reduce deprivation-induced water intake, saccharin intake and CMD intake, regression analyses indicated that the dose-response effects of Nor-BNI were more potent than naltrexone in inhibiting water and saccharin intake yielding similar ratios of 0.5 and 0.6, and almost as potent as naltrexone in inhibiting CMD intake yielding a ratio of 1.3 (see Table 3).

Table 3. Regression analysis summary table for deprivation-induced water intake, sucrose, saccharin and CMD intakes (ID_{50} in mM).

		Water	Sucrose	Saccharin	CMD
NTX	slope	-.058	-.102	-.062	-.062
	intercept	9.22	11.4	7.55	6.15
	ID_{50}	90	50	70	30
B-FNA	slope	.044	-.017	-.018	-.006
	intercept	9.53	12.98	10.14	7.56
	ID_{50}	110	450	470	660
	NTX ratio	1.2	8.4	6.6	22.7
N-BNI	slope	-.166	-.146	-.140	-.190
	intercept	13.03	11.15	10.64	11.75
	ID_{50}	50	20	40	40
	NTX ratio	0.5	0.3	0.6	1.3
Naloxonazine	slope	-.046	-.154	-.037	-.062
	intercept	14.97	19.77	11.79	11.96
	ID_{50}	280	120	280	160
	NTX ratio	3.0	2.3	4.0	5.4
DALCE	slope	-.068	-.125	-.069	-.011
	intercept	14.79	16.12	8.83	13.50
	ID_{50}	180	100	90	290
	NTX ratio	2.0	1.9	1.2	10.0
Naltrindole	slope	-.039	-.336	-.500	-.200
	intercept	10.03	15.41	12.46	14.48
	ID_{50}	260	60	30	90
	NTX ratio	2.8	1.2	0.5	3.1

Naltrexone: 1 ug=0.26 mM, 5 ug=1.32 mM, 20 ug=5.30 mM, 50 ug=13.25 mM.
B-FNA: 1 ug=0.20 mM, 5 ug=1.02 mM, 20 ug=4.08 mM.
N-BNI: 1 ug=0.14 mM, 5 ug=0.68 mM, 20 ug=2.72 mM.
Naloxonazine: 10 ug=1.54 mM, 20 ug=3.07 mM, 50 ug=7.69 mM.
DALCE: 10 ug=1.49 mM, 20 ug=2.97 mM, 40 ug=5.94 mM.
Naltrindole: 1 ug=0.22 mM, 5 ug=1.11 mM, 20 ug=4.44 mM.

The observations that baseline intake following water deprivation and CMD intake were inversely related to the magnitude of Nor-BNI's inhibitory effects may explain this apparent discrepancy between the analyses of variance and the regression data. It appears that rats with high baseline intakes for water deprivation and CMD are sensitive to Nor-BNI's inhibitory effects, while rats with low baseline intakes for water deprivation and CMD are not. However, this correlational analysis does not explain why Nor-BNI consistently failed to significantly inhibit saccharin intake, yet produced an ID_{50} similar to that of naltrexone.

B-FNA significantly inhibited deprivation-induced water intake, sucrose and CMD intake but not saccharin intake. These significant effects would suggest that B-FNA should exert potent inhibitory effects at low doses, yet, the regression analysis revealed very high ID_{50}s for all paradigms (see Table 3). This suggests that B-FNA effects are consistent, but of very small magnitude.

Naltrindole significantly inhibited saccharin intake but it failed to inhibit deprivation-induced water intake, sucrose and CMD intake as compared to vehicle buffer. The low ID_{50} (30 mM) and low ratio to naltrexone (0.48) seems to be consistent with these results (Table 3). However, the failure of naltrindole to alter sucrose intake would suggest that naltrindole should not exert potent inhibitory effects at low doses. It is therefore surprising to observe that the ID_{50} for naltrindole was only 60 mM which compares favorably with naltrexone (ratio of 1.2). The regression analyses for naltrindole-induced effects upon deprivation-induced water

intake and CMD intake were in agreement with this antagonist's failure to significantly alter these ingestive responses.

DALCE and naloxonazine only transiently stimulated sucrose intake but they failed to affect water, saccharin and CMD intake which seems to be consistent with the results revealed by regression analysis (Table 3).

These data revealed the following rank-order of opioid antagonist potencies in terms of the ID_{50} for each antagonist and for each form of intake:

Water intake: Nor-BNI (50 mM) > naltrexone (90 mM) > B-FNA (110 mM) > DALCE (180 mM) > naltrindole (260 mM) > naloxonazine (280 mM)

Sucrose intake: Nor-BNI (20 mM) > naltrexone (50 mM) > naltrindole (60 mM) > DALCE (100 mM) > naloxonazine (120 mM) > > B-FNA (450 mM)

Saccharin intake: naltrindole (30 mM) > Nor-BNI (40 mM) > naltrexone (70 mM) > DALCE (90 mM) > > naloxonazine (280 mM) > > B-FNA (470 mM)

CMD intake: naltrexone (30 mM) > Nor-BNI (40 mM) > naltrindole (90 mM) > > naloxonazine (160 mM) > > DALCE (290 mM) > > B-FNA (660 mM).

CHAPTER 8. GENERAL DISCUSSION

Naltrexone has been found effective in reducing the intake of all four ingestates. These results confirmed an opioid involvement in the modulation of water intake (e.g. Brown and Holtzman, 1979), and sucrose and saccharin intake (e.g. Cooper, 1983). They also demonstrated that endogenous opiates might be involved in the modulation of the intake of complex carbohydrates. The involvement of endogenous opioid system in ingestive behavior was first demonstrated by the general opioid antagonist, naloxone's ability (Holtzman, 1974) to decrease food and water intake in rats. Subsequent studies have found that naloxone decreased feeding in rats and mice under spontaneous (Jalowiec et al., 1981) and nocturnal (Brands et al., 1979; Jalowiec et al., 1981; Lowy et al., 1980) conditions and following food deprivation (Brands et al., 1979), norepinephrine (Morley et al., 1982), muscimol (Morley et al., 1981), 2-deoxy-D-glucose (Lowy et al., 1980), insulin (Lowy et al., 1980; Levine and Morley, 1981; Ostrowski et al., 1981; Rowland and Bartness, 1982), stress (mild tail-pinch and social conflict: Lowy et al., 1980; Morley & Levine, 1980), and hypothalamic stimulation-induced feeding (Jenck et al., 1986). Levine and co-workers (Levine et al., 1982) demonstrated that naloxone is more effective at reducing intake of sweetened solutions (0.2% saccharin or 2.0% sucrose) than unadulterated tap water. Opiate agonists are more effective at increasing the intake of palatable foods than less palatable foods (Shor-Posner et al., 1986). Thus, it was proposed that the opiates may have a role in the control of palatable food

consumption (Jackson & Cooper, 1985). In the present studies, naltrexone was found to be more effective in reducing CMD, sucrose and saccharin intake than it was in reducing water intake.

Sclafani and co-workers (Sclafani and Clyne, 1987; Sclafani and Mann, 1987) postulated that rats prefer complex carbohydrates to sucrose, glucose, fructose, and maltose solutions. They also observed that the preference threshold for polycose is 26 times lower than that for sucrose and maltose (Sclafani and Nissenbaum, 1987). Preference tests conducted with glucose polymers of different sizes further indicate that rats prefer polymers containing 4 to 8 glucose units relative to either smaller polymers or larger polysaccharides (Sclafani et al., 1987). In the present study naltrexone was much more effective in reducing the intake of CMD than it was in reducing the intakes of sucrose, saccharin and water. Since CMD is a medium size molecule as compared with the small molecule of sucrose, and CMD, sucrose and saccharin are more palatable than water, these results seem to confirm above-mentioned reports.

The mu_1-selective opioid antagonist, naloxonazine, failed to significantly affect any of the ingestates. Naloxonazine's failure could not be explained by a significant relationship to the baseline intake of the animals. In addition, its relatively high ID_{50}s and the lack of appreciable inhibition seem to exclude the involvement of the mu_1 opioid receptor in modulation of these forms of intake. In this light, any effects seen with B-FNA, a combined mu_1 and mu_2 antagonist, are assumed to be due to the

action on the mu_2 binding site. B-FNA significantly and dose-dependently reduced intakes of deprivation-induced water, sucrose, and CMD, but it failed to significantly affect intake of saccharin. On the other hand, regression analysis revealed high ID_{50}s for all of the liquids, suggesting that although B-FNA might produce significant reductions in the amount of solutions consumed, its involvement in modulation of the intake of these ingestates might be marginal. The involvement of the mu_2 receptor seems to be the strongest in regulation of deprivation-induced water intake. B-FNA significantly inhibited free-feeding (Arjune et al., 1990), deprivation-induced hyperphagia (Arjune et al., 1990; Simone et al., 1985), 2DG hyperphagia (Arjune and Bodnar, 1990; Arjune et al., 1990), and insulin hyperphagia (Beczkowska and Bodnar, 1991). Both forms of glucoprivic feeding, in contrast to free- and deprivation-induced feeding, were not affected by naloxonazine, which illustrates the involvement of the mu_2 opioid receptor in these forms of the regulatory challenge. These observations and the results of the present study suggests that the mu_2 opioid receptor might be involved in the modulation of food and water intake in some, but not all, ingestive situations under regulatory challenge. Also, B-FNA's inhibitory effects upon CMD intake were most potent in animals with high baseline CMD intake and ineffective in animals with low baseline CMD intake. These results suggests that mu_2 opioid receptor, in addition to being involved in modulation of deprivation-induced water intake, might play a secondary role in modulation of an intake of complex carbohydrates.

The kappa-selective opioid antagonist, Nor-BNI, significantly and dose-dependently inhibited the intake of sucrose, but it failed to affect deprivation-induced water intake, and saccharin and CMD intakes. The facts that Nor-BNI's effects were strongest in animals with low baseline sucrose intake, and that it was found ineffective in reducing saccharin intake, suggest that kappa receptor plays a primary role in opioid modulation of an intake of simple sugars. A reverse relationship between the effects of the antagonist and the baseline intake of the animals was found for deprivation-induced water intake and for CMD intake. Nor-BNI was most effective in reducing intakes in animals with high baseline deprivation-induced water intake and high baseline CMD intake. These findings suggest that there is a secondary kappa mediation of deprivation-induced water intake, and complex carbohydrate intake.

In addition, Nor-BNI was as effective as naltrexone in inhibiting free feeding (Arjune et al., 1991), and feeding induced by kappa-selective (U50,488H), mu-selective (DAMGO), and delta-selective (DSLET) agonists (Levine et al., 1990), indicating that the kappa receptor is an integral component in the synaptic chain of events that modulates of feeding stimulated by all opioid receptor subtype agonists. Further, Nor-BNI was more effective than general opioid antagonists in inhibiting high-fat intake, but it was only marginally involved in deprivation-induced food and water intake (Islam and Bodnar, 1990; Arjune et al., 1991; Chapter 3). It would seem advantageous for an animal in an energy crisis situation to ingest simple sugars,

which are absorbed almost immediately into the blood stream, as well as fat, which is quickly moved to replace fat stores in adipose tissue depleted by deprivation. However, the above evidence seems to suggest that the kappa receptor plays only a secondary role in deprivation-induced ingestive behavior. Studies evaluating opioid modulation of macronutrient selection in both freely-feeding and food-restricted conditions typically have employed either the general opioid antagonist, naloxone or the prototypical opiate agonist, morphine (e.g., Marks-Kaufman, 1982; Marks-Kaufman and Kanarek, 1990; Shor-Posner et al., 1986; Bhakthavatsalam and Leibowitz, 1986). Morphine is somewhat selective for mu receptors, but can bind to delta and kappa receptors as well (see review: Pasternak and Wood, 1986). To establish clearer relationships for the kappa receptor between macronutrient diet selection and deprivation status of animals, studies employing selective kappa agonists are necessary.

The $delta_2$ antagonist, naltrindole, and, to a lesser extent, the $delta_1$-selective opioid antagonist, DALCE, significantly inhibited saccharin intake, but failed to significantly alter deprivation-induced water intake, as well as intake of sucrose and CMD solutions. Regression analysis revealed that, although naltrindole failed to significantly reduce sucrose intake, naltrindole's potency in reducing sucrose intake (ID_{50}: 60 mM) was similar to that observed for naltrexone ID_{50}: 50 mM), and similar to naltrindole's potency in reducing saccharin intake (ID_{50}: 30 mM). These findings suggest that perhaps naltrindole would have been found effective in inhibiting sucrose

intake had a higher dose been administered. Since saccharin and sucrose share one characteristic, a sweet taste, and neither water nor CMD have a sweet taste (Sclafani, 1987; 1991), is possible that $delta_2$ opioid receptor might be involved in mediation of intake of foods that taste sweet. This is quite striking since delta receptor antagonists have produced minimal effects in altering other forms of ingestive situations (Arjune et al., 1991; Beczkowska and Bodnar, 1991; Islam and Bodnar, 1990; Jackson and Sewell, 1985). Future studies employing higher doses of naltrindole might further explain the role of delta opioid receptors in these forms of ingestion.

Since B-FNA significantly inhibited deprivation-induced water intake and intake of sucrose and CMD solutions, and the mu_1 antagonist failed to significantly alter these forms of intake, the observed results indicate mu_2 mediation of these effects. However, the low potency and limited degree of inhibition of B-FNA suggest that the mu_2 opioid receptor may be more involved in long-term regulation of ingestion possibly related to challenge situations and body weight maintenance rather than regulation of mechanisms related to palatability. The kappa opioid receptor appears to be involved primarily in the mediation of intake of simple sugars, and secondarily in the intake of complex carbohydrates. Sham-feeding studies (Kirkham and Cooper, 1988a,b) employing naloxone suggest that the post-ingestive consequences are not required for the opioid modulation of ingestion. Since Nor-BNI failed to alter intake of saccharin solution, which is palatable but not nutritive,

kappa involvement in palatable intake appears to be limited to the mediation of ingestion of foods that are characterized by post-ingestive consequences. Finally, the roles of delta receptor subtype antagonists appear to be limited to the modulation of intake of substances with sweet tastes.

The involvement of the endogenous opioid system in the mediation of ingestion has been confirmed using a wide array of pharmacological and physiological techniques (see reviews: Levine et al., 1985; Morley and Levine, 1983). It has been postulated that the primary mechanism of opioid modulation of feeding behavior is through its influence upon palatability (Cooper et al., 1985). A primary goal of the present dissertation was to characterize further the role of opioid receptor subtypes in ingestion of palatable foods.

Although agonist studies suggested a role for the delta receptor in different forms of ingestion, antagonist studies have typically failed to discern consistent effects. These data have been interpreted to suggest that whereas other opioid receptor subtypes are in the direct synaptic chain that is essential for the expression of feeding behavior under different circumstances, the delta receptor might act as a modulator, fine-tuning those ingestive responses in which delta agonists stimulate intake (Arjune et al., 1991). Given that saccharin intake appears to be stimulated by purely orosensory mechanisms, the findings that delta antagonists inhibit saccharin intake confirm their role in fine-tuning modulation of ingestive behavior. Evidence has accumulated that the mu receptor was involved in spontaneous intake,

deprivation-induced intake and intake following regulatory challenges. The present findings indicate that blockade of the mu, but not the mu_1 receptor, significantly inhibits deprivation-induced water intake, and intake of simple and complex carbohydrate solutions. However, the low potency and limited degree of inhibition by B-FNA as compared to naltrexone in paradigms related to palatability suggest that mu-mediated actions upon ingestive behavior are limited to the instances regulatory challenge rather than the mediation of palatability.

In summary, the present dissertation confirmed the involvement of the endogenous opioid system in the mediation of deprivation-induced water intake as well as intake of sucrose and saccharin solutions. Thus, central administration of naltrexone significantly inhibited palatable intake that was characterized by post-ingestive consequences (sucrose) and palatable intake that had no nutritive value (saccharin). This dissertation expanded endogenous opioid involvement in macronutrient intake to control over intake of polysaccharide. In all of the ingestive situations, general opioid antagonism interfered with the maintenance, rather than the initiation of ingestive behavior. It is not possible at the present time to determine whether any opioid antagonist-induced decreases observed in the intake of sucrose, saccharin and CMD were due to the fact that the animal enjoyed the ingestate less or that the animal was getting less input. Anatomically, there is an opioid substrate to support primarily sensory mechanism with the predominant role of hypothalamus, trigeminal nerve, and the dorsal vagus. However, more integrative

areas, like thalamus and cortex, also may play a role in processing of ingestive information. Further studies need to explore this question. In addition, it should be pointed out that this dissertation is primarily a pharmacological study evaluating opiate action, therefore, an array of general and existing selective compounds were employed that cover all of the known opioid receptor subtypes. In keeping with this pharmacological analysis, full dose-response curves were done for all antagonists employed. To establish the precise intervals and duration of antagonists effects temporal analysis was performed. Finally, to determine opiate action across an array of ingestates, the four variables of water-deprivation, sucrose, saccharine and CMD were compared in regression analysis. However, because of the large parametric analysis of the variable stated above, only one concentration of the ingestates (10% sucrose, 0.1% saccharine, and 10% CMD) were employed. These concentrations were selected to obtain reliable, consistent and large enough intake of a given fluid to observe inhibitory action of the antagonists. There is one potential flow in this reliance upon only one concentration of the given ingestates. It is conceivable that any observed opioid antagonist effects upon a given ingestate may be limited to the concentration that way employed and that a pattern of effects could change as a function of other concentrations. Further studies need to investigate these questions.

A major thrust of previous studies (see reviews: Cooper et al., 1985; Levine et al., 1985; Morley and Levine, 1983; Morley et al., 1985) hypothesized that: a) palatability is a major factor in determining opioid involvement in ingestion and b)

the kappa receptor is the major opioid receptor subtype involved in the modulation of palatability. Both of these hypotheses must be re-evaluated and the role of the opioid system in the mediation of feeding responses must be re-defined in terms of the present findings. First, the kappa antagonist, Nor-BNI, was the most effective antagonist in inhibiting sucrose intake, a prototypical ingestate employed in studies of palatability, but it was ineffective in altering saccharin intake. The major difference between sucrose and saccharin is the respective presence or lack of post-ingestive consequences. Therefore, these data suggest that the kappa participation in the modulation of intake must involve post-ingestive factors. Second, post-ingestive events may vary as a function of the type of macronutrient that is consumed. Hence, the potent effects of Nor-BNI upon sucrose intake (Chapter 4) and high-fat intake (Islam and Bodnar, 1990) but its relative ineffectiveness in altering intake of a complex carbohydrate solution, indicate that the kappa receptor's role in modulation of ingestion may be macronutrient specific to simple sugars and fat. In addition, the presence of two distinct taste systems, sugar-sensitive and starch-sensitive, (Sclafani, 1987, 1991), appear to suggest that the kappa receptor is intimately involved in the mediation of the former system, but not the latter system, again suggesting kappa involvement in the modulation of ingestion of simple sugars. Hence, it would appear that kappa modulation is quite striking in a) feeding situations with distinct post-ingestive consequences, and b) the ingestion of simple sugars that have immediate, though short-term ingestive consequences.

A categorization of opioid control of ingestion appears to involve the delta receptor in fine-tuned modulation of orosensory signals, the kappa receptor in immediate ingestive consequences, and the mu receptor in longer-term regulatory consequences and weight maintenance. In the light of the above mentioned evidence, it seems that the role of the opioid system in mediation of feeding responses, in addition to the modulation of palatability, should be expanded to include its involvement in post-ingestive mechanisms and macronutrient selection.

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