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Opioid receptor involvement in food intake and body weight maintenance: Role of the mu-1 binding site

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OPIOID RECEPTOR INVOLVEMENT IN FOOD INTAKE AND BODY WEIGHT MAINTENANCE: ROLE OF THE MU-1 BINDING SITE

by

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Abstract

OPIOID RECEPTOR INVOLVEMENT IN FOOD INTAKE AND BODY WEIGHT MAINTENANCE: ROLE OF THE MU-1 BINDING SITE

by

Phyllis E. Mann

Advisor: Professor Richard J. Bodnar

Opiate receptor agonists and antagonists influence feeding behavior. Agonists of each opioid receptor subtype increase feeding. By comparing naloxone, a short-acting opioid receptor antagonist with naloxonazine, an irreversible and highly-selective antagonist of the mu-1 binding site, it is possible to categorize opioid modulation into mu-1-mediated and non-mu-1-mediated actions. The mu-1 site has been implicated in the opioid component modulating free-feeding and deprivation-induced feeding but not glucoprivic feeding. This dissertation further categorizes different feeding models by examining naloxone and naloxonazine effects upon opioid-induced feeding and upon chronic body weight maintenance and food intake in

adult, adolescent and dietary obese rats.

The role of the mu-1 binding site in opiate receptor agonist hyperphagia was examined first in adult male rats. Morphine (5 mg/kg, s.c.) hyperphagia was blocked by naloxone (10 mg/kg, s.c. or i.v.) but was potentiated by naloxonazine (10 mg/kg, i.v.). Only naloxone blocked ethylketocyclazocine (EKC) hyperphagia at 2 mg/kg (s.c.) whereas neither blocked EKC hyperphagia at 5 mg/kg. Dynorphin (10 ug, i.c.v.) and D-ala^2-D-leu^5-enkephalin (DADL; 10 ug, i.c.v.) hyperphagia was blocked by naloxone but was unaffected by naloxonazine.

The effects of daily administration of naloxone and naloxonazine in three types of maturational or dietary situations was examined next. In adult rats, naloxone and naloxonazine reduced body weight by 4% and 7%, respectively and food intake by 13% and 22%, respectively over 14 days. In adolescent rats, naloxone and naloxonazine reduced body weight gain by 33% and 53%, respectively and food intake by 14% and 27%, respectively over 14 days. In contrast, neither chronic naloxone nor naloxonazine treatment altered body weight or food intake of rats previously made obese by a "cafeteria" diet.

The use of naloxone and naloxonazine has shown that the mu-1 binding site may be implicated in free-feeding, deprivation-induced feeding and chronic food intake and body weight maintenance sugges-

ting the involvement of the mu-1 site in "long-term" conditions. In contrast, glucoprivic feeding and opioid-induced feeding may be mediated by non-mu-1 binding sites suggesting that other opioid receptor subtypes may be involved in either "short-term" regulatory challenges or stimulatory conditions.

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Introduction

Recent developments in the research on opiate receptors, opiate receptor subtypes and endogenous and exogenous opioid agonists and antagonists has led to new insights in their role in physiological functions and behavior. Biochemical evidence has indicated the existence of two distinct mu opiate receptors termed mu-1 and mu-2. The mu-1 receptor appears to be a common high affinity binding site for variety of opiate drugs and opioid peptides. The development of long-lasting opiate receptor antagonists such as naloxonazine specific for the mu-1 binding site has allowed researchers to pinpoint whether this site is involved in specific opiate mediated behaviors. The mu-1 binding site has been implicated in opiate modulation of supraspinal analgesia, catalepsy, hypothermia, prolactin release and acetylcholine turnover, but not in opiate modulation of spinal analgesia, respiratory depression, sedation, bradycardia, growth hormone release, dopamine turnover and many components of physical dependence. Evidence also suggests that opiates are involved in ingestive behavior. While agonists of each opiate receptor subtype stimulate feeding, naloxone, a general opiate receptor antagonist inhibits feeding and blocks opiate agonist effects upon feeding. Naloxonazine blocks free-feeding and deprivation-induced feeding but not feeding induced by gluco-privation. The present series of experiments evaluated the role of

the mu-1 binding site in the following feeding models: acute feeding induced by different opiate receptor agonists including morphine, ethylketocyclazocine (EKC), dynorphin and D-ala^2-D-leu^5-enkephalin (DADL), and chronic maintenance of body weight and food intake in adult, adolescent and dietary-obese rats (please see the Appendix for a Table of Abbreviations).

The following sections provide background information pertaining to a) opiate receptors and endogenous opioid peptides, b) the high-affinity (mu-1) opioid binding site, and c) the relationship between opiates and ingestive behavior.

A. Opiate Receptors and Opioid Peptides

Pharmacologists postulated the existence of opiate receptors in the 1940's upon the realization that opiate narcotic analgesics such as morphine possess structural and stereospecific properties that are closely related to their action. In 1973, several laboratories (Pert & Snyder, 1973a; Simon, Hiller, & Edelman, 1973; Terenius, 1973) reported stereospecific opiate binding in rat brain tissue. Opiate binding is saturable, highly specific and possesses high affinity (Pert & Synder, 1973b) with high correlations between binding affinities and pharmacological potency of opiates (Creese & Snyder, 1975; Pert & Snyder, 1973b; Stahl, van Bever, Janssen, & Simon, 1977). Opiate receptors were found to be located in the central nervous system (CNS), in the innervation of the smooth muscles of the gut and vas deferens, and in the pituitary and adrenal glands of

vertebrates including humans (Atweh & Kuhar, 1977a,b,c; Hiller, Pearson, & Simon, 1973; Kuhar, Pert, & Snyder, 1973; Palacios & Maurer, 1984; Pasternak, Goodman, & Snyder, 1975; Pert, Aposhian, & Snyder, 1974; Stefano, Kream, & Zukin, 1980). The regional distribution of opiate receptors in the CNS is not uniform but concentrated in particular areas, including the frontal cortex, limbic system, basal ganglia, medial thalamus, periaqueductal and periventricular gray regions, nucleus raphe magnus and the substantia gelatinosa of the dorsal horn of the spinal cord.

The discovery of the opiate receptor in the CNS was followed by the search and subsequent isolation of endogenous opioid ligands. Hughes (1975) found a substance in extracts of pig brain which had opiate-like actions in the mouse vas deferens and in the guinea pig ileum bio-assays. Terenius and Wahlstrom (1975) isolated a factor from rat and calf brain that could compete with labeled opiates for receptor binding. The active factor was purified and characterized as two pentapeptides, H-Tyr-Gly-Gly-Phe-Met-OH (met-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (leu-enkephalin) (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975). Opioid activity was also found in two compounds (alpha- and gamma-endorphin) isolated from pig hypothalami and pituitary glands which were present in the hormone beta-lipotropin (Ling, Bargus, & Guillemin, 1976). The C-terminal fragment of beta-lipotropin (beta-endorphin) also possessed opioid activity (Bradbury, Smyth, Snell, Birdsall, & Hulme, 1976; Cox, Goldstein, & Li, 1976). Dynorphin, a potent opioid, was subsequently

isolated from pituitary extracts (Goldstein, Tachibana, Lowney, Hunkapiller, & Hood, 1979). Further studies using messenger RNA techniques indicated that proopiomelanocortin was the precursor for beta-endorphin as well as for adrenocorticotrophic hormone (ACTH) and the melancoyte stimulating hormone (MSH) families of peptides; proenkephalin was the precursor for met-enkephalin and prodynorphin was the precursor for dynorphin and alpha-neo-endorphin (see review: Akil, Watson, Young, Lewis, Khachaturian, & Walker, 1984).

The existence of different subtypes of opiate receptors was postulated based upon the heterogeneity of endogenous opioid peptides and the differing pharmacological effects and potencies for different opiates. Martin and co-workers described three kinds of opiate receptor subtypes based on their pharmacological studies in chronic spinal dogs (Martin, Eades, Fraser, & Wikler, 1964; Martin, Eades, Thompson, Huppler & Gilbert, 1976). The opiate receptor agonists morphine, ketocyclazocine and SKF-10,047 (N-allylnormetazocine) produced different physiological and behavioral effects when injected into nondependent spinal dogs which failed to show cross-tolerance, and did not precipitate or prevent abstinence effects when injected into dependent spinal dogs. The three opiate receptors were named according to the prototypic agonist: mu for morphine, kappa for ketocyclazocine and sigma for SKF-10,047. Receptor heterogeneity was also found in bioassay systems in vitro. Using the guinea pig ileum and the mouse vas deferens assays, Lord and co-workers found that the depression of electrically-induced contractions of the guinea pig

ileum was more sensitive to morphine than to enkephalins (mu: Lord, Waterfield, Hughes, & Kosterlitz, 1977). The reverse occurred in the mouse vas deferens: enkephalins were more potent than morphine in suppressing contractions (delta: Lord et al., 1977). Receptor binding studies and cross-protection experiments provide further confirmation that different opiate receptor populations existed. Enkephalins compete better against labelled enkephalins than labelled opiates for receptor binding and vice versa (Lord et al., 1977; Chang & Cuatrecasas, 1979; Simon, Bonnet, Crain, Groth, Hiller, & Smith, 1980). Opiates such as morphine protect the morphine binding site from inactivation better than enkephalins, whereas enkephalins protect the enkephalin binding site better than morphine (Robson and Kosterlitz, 1979; Smith & Simon, 1980). Cross-protection studies have also confirmed the existence of kappa receptors. Ethylketocyclazocine (EKC) is more effective than morphine in protecting labelled EKC binding sites from inactivation by phenoxybenzamine (Kosterlitz & Leslie, 1978; Kosterlitz & Paterson, 1980). Finally, regional distribution studies using receptor autoradiography indicated that the ratio between mu and delta receptors varies as a function of brain region. For example, the thalamus and hypothalamus have more mu receptors than delta receptors, whereas the frontal cortex and striatum have equal concentrations of mu and delta receptors (Chang, Cooper, Hazum, & Cuatrecasas, 1979; Goodman, Snyder, Kuhar, & Young, 1980; Herkenham & Pert, 1980; Simantov, Childers, & Snyder, 1978).

Endogenous opioids act like opiate agonists in both binding assays and bioassays and have similar pharmacological effects when administered in vivo (e.g. analgesia, catatonia, respiratory depression, development of tolerance and physical dependence). They are found in all vertebrate species studied (e.g. Bloom, Battenberg, Rossier, Ling, & Guillemin, 1978; Watson, Richards, & Barchas, 1978) and in some invertebrates (Alumets, Kahanson, Sundler, & Thorell, 1979; Zipser, 1980). The regional distribution of endogenous opioids in general is similar to the distribution of opiate receptors with certain exceptions (Lewis, Khachaturian, & Watson, 1982; Pasternak et al., 1975a; Quirion, Bowen, Herkenham, & Pert, 1982). The enkephalins are extensively distributed as interneurons in the telencephalon (e.g cerebral cortex, limbic system), diencephalon (hypothalamus and lateral geniculate body), midbrain (e.g. colliculi, periaqueductal gray), pons and medulla (e.g. parabrachial and dorsal tegmental nuclei, nucleus tractus solitarius and raphe), and the dorsal horn of the spinal cord (Khachaturian, Lewis, Hollt, & Watson, 1983; McGinty, van der Kooy, & Bloom, 1984). Beta-endorphin cell bodies are mainly found in the arcuate region of the hypothalamus and nucleus tractus solitarius (Bloom et al., 1978; Schwartzberg & Nakane, 1983; Watson, Barchas, & Li, 1977). The immunoreactive fiber system of beta-endorphin cells in the arcuate nucleus has extensive projections to the periventricular thalamus, periaqueductal gray, medial amygdala, locus coeruleus, and raphe magnus (Bloom et al., 1978; Watson et al., 1977). Immunoreactive dynorphin cell bodies and fiber systems

are located in the cerebral cortex, limbic system, hypothalamus, periaqueductal gray, parabrachial nucleus, nucleus tractus solitarius, lateral reticular nucleus, spinal trigeminal nucleus, and spinal cord dorsal horn (McGinty et al., 1984; Weber & Barchas, 1983; Khachaturian, Watson, Lewis, Cox, Goldstein, & Akil, 1982; Vincent, Hokfelt, Christensson, & Terenius, 1982).

In summary, there are three families of opioid peptides derived from one of three precursor molecules: proopiomelanocortin (beta-endorphin), proenkephalin (met- enkephalin) and prodynorphin (dynorphin). These peptides appear to interact with at least four subtypes of opioid receptors: mu, kappa, delta and epsilon. The mu receptor subtype can be further classified into mu-1 and mu-2 based on the following biochemical and pharmacological evidence.

B. The High-Affinity (Mu-1) Opioid Binding Site.

When labelled opiate drugs with very high specific activity became available, curvilinear Scatchard plots and biphasic displacement curves of opiate binding were reported (Lord et al., 1977; Pasternak & Snyder, 1975). Biphasic Scatchard plots suggest the existence of at least two populations of binding sites: high-affinity and low-affinity. The high-affinity opioid binding site is more sensitive to the effects of sodium, enzymes and protein-modifying reagents than the low-affinity site (Pasternak, Snowman, & Snyder, 1975b; Pasternak, Wilson, & Snyder, 1975; Pasternak & Snyder, 1974; Pasternak & Snyder, 1975; Creese, Pasternak, Pert, & Snyder,

1975; Wilson, Pasternak, & Snyder, 1975). The high-affinity and low-affinity sites were initially thought to either represent the agonist or antagonist forms of the receptor (Pasternak & Snyder, 1975) or the different sites represented binding to different receptors (e.g., high-affinity: delta; low-affinity: mu) (Lord et al., 1977). The development of highly selective, irreversible antagonists for the high-affinity binding site (e.g., naloxazone) provided further evidence for the existence of at least two distinct binding sites (Pasternak, 1980; Pasternak, Childers, & Snyder, 1980a,b; Zhang, Chang, & Pasternak, 1981). Naloxazone blocked high-affinity binding of radiolabelled morphine in mice without affecting low-affinity binding and attenuated morphine analgesia without altering morphine lethality (Pasternak et al., 1980a,b). Pasternak and co-workers termed this high-affinity binding site mu-1 and the low-affinity binding site mu-2. Naloxazone also blocked the high-affinity binding of radiolabelled enkephalins, beta-endorphin, EKC and SKF 10,047 but had negligible effects on the low-affinity binding characteristics of these ligands (Hazum, Chang, Cuatrecasas, & Pasternak, 1981; Johnson, Houghten, & Pasternak, 1982; Pasternak, 1980; Wolozin, Nishimura, & Pasternak, 1982; Zhang & Pasternak, 1980). Further, differential regional localizations of high-affinity and low-affinity binding sites were found in the brain and spinal cord (Zhang & Pasternak, 1980). Naloxazone produced greater inhibition of binding in the hypothalamus and spinal cord (mu-1 sites) than in the frontal cortex, pons medulla and midbrain (mu-2). Auto-

radiographic techniques have confirmed the high levels of mu-1 binding in areas known to be involved in analgesia including the periaqueductal gray, medial thalamus and median raphe (Goodman & Pasternak, 1985). Mu-1 sites were also found in the nucleus accumbens, striatum, hypothalamus, medial habenula and the medial septum. Developmental differences in the appearance of high-affinity and low-affinity binding sites further substantiated the existence of two distinct binding sites. During the first two postnatal weeks in rats, low-affinity binding of labelled morphine remains relatively constant, whereas high-affinity binding increases three-fold (Pasternak, Zhang, & Tecott, 1980; Zhang & Pasternak, 1981a). The changes in binding correlate with morphine's analgesic and respiratory effects: morphine analgesia increases 40-fold in the first two weeks of life while morphine effects on respiratory depression remain constant. Phylogenetic differences in the proportion of high-affinity to low-affinity binding are also observed (Buatti & Pasternak, 1981). Naloxazone blocked high-affinity opiate binding in rat and turtle to the same degree, yet was less effective in frog and was ineffective in goldfish. Based on the above evidence, Wolozin and Pasternak (1981) postulated three classes of receptors for opiates and enkephalins: a) mu-1, a high-affinity binding site that binds opiates and enkephalins equally, b) mu-2, a low-affinity site that binds morphine and its analogs more potently than enkephalin analogs and c) delta, a low-affinity site that binds enkephalin analogs more potently than morphine.

Naloxazone and naloxonazine, hydrazone derivatives of naloxone (Hahn & Pasternak, 1982), have been invaluable tools in trying to understand the physiological and pharmacological relevance of the mu-1 receptor and whether it mediates particular opioid actions. Supraspinal opiate and opioid peptide analgesia appear to be mediated by the mu-1 receptor (Zhang & Pasternak, 1981b). If naloxazone was administered 24 h before testing to eliminate unbound drug, it blocked analgesia induced by morphine, D-ala-met-enkephalinamide, and beta-endorphin similarly, thereby suggesting a common receptor mechanism. Catalepsy induced by high doses of morphine was also blocked by naloxazone administered 24 h earlier (Ling & Pasternak, 1982). In contrast, naloxazone failed to affect endotoxic shock (Holaday, Pasternak, D'Amato, Ruvio, & Faden, 1983). The use of naloxonazine demonstrated receptor dissociation of morphine analgesia from physical dependence (Ling, MacLeod, Lee, Lockhart, & Pasternak, 1984). Administration of naloxonazine 24 h prior to chronic infusion with morphine significantly reduced morphine analgesia, whereas withdrawal signs were virtually unaffected (e.g., ptosis, tachypnea and weight loss). Naloxonazine also eliminated morphine analgesia in adult rats without affecting the respiratory depressant effects of morphine (Ling, Spiegel, Lockhart, & Pasternak, 1985; Ling, Spiegel, Nishimura, & Pasternak, 1983).

By comparing the effects of naloxazone and naloxonazine with naloxone, it is possible to separate out which opioid effects are mu-1 mediated and which are non-mu-1 mediated. Reversal of an opioid-mediated effect by naloxone defines opioid action. Reversal

by both naloxone and naloxazone or naloxonazine defines involvement of the mu-1 binding site. Reversal by naloxone but not by either naloxazone or naloxonazine defines a non-mu-1 opioid action. This approach has made it possible to differentiate mu-1 involvement in supraspinal morphine analgesia (Ling & Pasternak, 1983), morphine catalepsy (Ling & Pasternak, 1982), release of prolactin by morphine (Spiegel, Kourides, & Pasternak, 1982), acetylcholine turnover and hypothermia, and non-mu-1 involvement in respiratory depression (Ling et al., 1985), growth hormone release (Spiegel et al., 1982), bradycardia, sedation, dopamine turnover (Wood & Pasternak, 1983) and inhibition of the electrically-stimulated guinea pig ileum (Gintzler & Pasternak, 1983; for review, see Pasternak & Wood, 1986).

The existence of opiate receptors and endogenous opioid peptides led to speculation about the physiological significance of these systems. Opiate drugs have been known for some time to produce a variety of effects such as analgesia, sedation, catalepsy, respiratory depression, bradycardia and pupil constriction. Endogenous opioids are thought to be involved in many physiological and behavioral functions including the perception and reaction to noxious stimuli, the response to stress, learning and memory, cardiovascular responses, thermoregulation and ingestion (see review: Olson, Olson, & Kastin, 1985b). The following section provides a brief review of opioid involvement in the latter function.

C. Ingestive Behavior

Many factors are involved in the control of food intake. Most

of the work that had been done in this area examined specific metabolites of food such as glucose, amino acids and fatty acids or looked at gastrointestinal hormones such as insulin and glucagon. Other investigators have looked at central nervous system factors involved in ingestive behavior: the role of neurotransmitters (e.g. norepinephrine, dopamine and serotonin) and the importance of specific brain areas such as the ventromedial hypothalamus (VMH), the lateral hypothalamus (LH) and the caudal hindbrain. In addition to the involvement of the central nervous system there are peripheral factors that must be taken into account. The mouth, stomach, small intestine and liver may be just as important as the brain in controlling ingestive behavior. The issue becomes even more complex when you consider the psychological aspects of food intake. Appetite is not only the sensation of hunger but can involve hedonic qualities associated with the sight and smell of food. What makes an animal or a person stop eating is also complicated and is not just based on the sensation of a full stomach. Satiety can also involve the novelty or the aversiveness of the food.

There are three main theories describing which factors are important in the central regulation of food intake: the glucostatic, lipostatic and aminostatic hypotheses. The glucostatic hypothesis originally stated that it is the level of glucose in the blood (which the brain monitors) that regulates food intake. It was then thought that it is not the blood glucose level but the degree of glucose utilization that is important (Mayer, 1955) since diabetic patients who have very high levels of blood glucose are always hungry. This

hypothesis has been criticized since it has been impossible to find an area in the brain which responds to glucose injections. Hungry animals do not stop eating if glucose is injected into areas of the brain (Balagura, 1973). The lipostatic hypothesis is based on the observation that animals can regulate their fat deposits and that diabetic rats respond to changes in the composition of fat in their diets. Rats that have been treated with alloxan, a diabetogenic agent, will alter their intake to correspond with changes in the fat content of their diets (Friedman, 1978). They will not respond to changes in carbohydrate concentration. Amino acids are also produced as a result of food intake and are thought to be involved in ingestive behavior (Mellinkoff, Frankland, Boyle, & Greipel, 1956) although the aminostatic hypothesis has not been as well researched as the other two.

The mouth may play a part in the regulation of food intake by signaling satiety. Esophagotomized dogs will stop eating a meal even though the food has not reached their stomach (Janowitz & Grossman, 1949). There is evidence that the stomach has both nutrient and stretch receptors that monitor the content and volume of food that enters which would also signal satiety (Deutsch, 1978). The liver, too, is thought to be important in food intake. Injections of glucose into the hepatic portal vein but not the jugular vein produces satiety (Russek, 1971) and blocking the vagal afferent from the liver to the brain decreases feeding (Penaloza-Rojas & Russek, 1963).

The central nervous system is also involved in the control of

ingestive behavior. Evidence for this has come from lesion studies of specific brain areas and pathways and from microinjections of a variety of substances into the brain which either stimulate or inhibit feeding. The ventromedial nucleus of the hypothalamus (VMH) and the lateral hypothalamus (LH) were thought to be the "satiety center" and the "feeding center", respectively, based on early studies that lesioned these areas (Hetherington & Ranson, 1939; Anand & Brobeck, 1951). Further evidence has shown that is not the lesioned nuclei themselves that explain the effects that were observed but the fiber pathways that pass through them (Kapatos & Gold, 1973; Ziegler & Karten, 1974). The caudal hindbrain has also been implicated in feeding behavior (Ritter, 1984). This area of the brain contains the motor nuclei of the vagus nerve and the solitary tract nuclei which receive visceral afferents from the gastrointestinal tract and the area postrema, a circumventricular organ. Rats that receive lesions of the area postrema and the adjacent nuclei of the solitary tract lose weight and maintain their body weight at lower levels than controls (Edwards & Ritter, 1981).

Evidence is accumulating that a variety a neurotransmitters and neuromodulators are involved in food intake. Historically, monoamines have been implicated in feeding behavior having both stimulatory and suppressive actions (Hoebel, 1977; Leibowitz, 1980). Norepinephrine when injected into the paraventricular nucleus of the hypothalamus (PVN) reliably stimulates feeding (Woods & Leibowitz, 1985) usually at a shorter latency than opioid effects. Dopamine agonists and antagonists can either increase or suppress feeding

depending on the locus of injection (Leibowitz, 1980). The dopamine agonist bromergocryptine induced feeding that is inhibited by the dopamine antagonist haloperidol and by naloxone (Morley et al., 1982a). Serotonin and related compounds are well known regulators of appetite (Blundell, 1979), as are the gut peptides (Baile, McLaughlin, & Della-Fera, 1986; Morley, Levine, Gosnell, & Krahn, 1985). The pancreatic polypeptide family which includes human pancreatic polypeptide, neuropeptide Y and peptide YY, have been known to stimulate food intake (Clark, Kalra, Crowley, & Kalra, 1984; Morley & Levine, 1984). Inhibitors of food intake include cholecystokinin (Baile et al., 1986), calcitonin (Freed, Perlow, & Wyatt, 1979; Levine & Morley, 1981), bombesin (Kulkosky, Gibbs, & Smith, 1982; Morley & Levine, 1981a) and somatostatin (Aponte, Leung, Gross, & Yamada, 1984).

In the last several years, a growing number of reports have shown that opiates and opioid peptides are involved in food intake under a wide variety of conditions (Morley, Levine, Yim, & Lowy, 1983; Reid, 1985). The following section will describe some of the research that has been accomplished in this field.

D. Ingestive Behavior and Opioid Peptides

The first definitive evidence for the involvement of opiates in ingestive behavior was obtained using the short-acting opiate receptor antagonists naloxone and naltrexone. Holtzman (1974) found that systemic administration of naloxone reduced food intake in rats previously deprived of food for 48 h. This anorectic effect has been

widely replicated and occurs in rats (e.g. Brown & Holtzman, 1979; Frenk & Rogers, 1979), mice (Brown & Holtzman, 1979), rabbits (McCarthy, Dettmar, Lynn, & Sanger, 1981), cats (McCarthy et al., 1981), sheep (Baile, Keim, Della-Fera, & McLaughlin, 1981), fish (Christ, 1984), pigeons (Deviche, Helmer, & Schepers, 1984) and humans (Morley, Gosnell, & Levine, 1984) but not in slugs (Kavaliers, Rangeley, Hirst, & Teskey, 1986). Naloxone and naltrexone also reduce food intake in non-deprived rats (Brands, Thornhill, Hirst, & Gowdey, 1979; Carey, Ross, & Enns, 1981; Cooper, 1980) and decrease water intake under both deprived and non-deprived conditions (e.g. Brown & Holtzman, 1979, 1981; Frenk & Rogers, 1979; Frenk & Rosen, 1979). These opiate receptor antagonists have also been shown to reduce the hyperphagic effects of 2-deoxy-D-glucose (Ostrowski, Rowland, Foley, Nelson, & Reid, 1981; Simone, Bodnar, Goldman, & Pasternak, 1985; Thompson, Welle, Lilavivat, Penicaud, & Campbell, 1982), mild tail pinch (Lowy, Maickel, & Yim, 1980; Morley & Levine, 1980; but see Ostrowski et al., 1981) and hypothalamic brain stimulation (Carr & Simon, 1984).

If opiate receptor antagonists reduce feeding, it might be expected that opiate receptor agonists should potentiate feeding. Morphine was historically described as an inhibitor of food intake, but Martin and co-workers initially noted that dependent animals displayed increases in food intake (Martin, Wikler, Eades, & Pescor, 1963). Sanger and McCarthy (1980) showed that morphine's ability to either stimulate or inhibit food intake depended upon the relative

deprivation state prior to injection. Systemic administration of morphine increased both food and water intake in non-deprived animals, yet decreased food and water intake in animals that had been previously deprived for 24 h. When rats are mildly food deprived (e.g., 5 h), systemic administration of morphine increased intake in the first 4 h after deprivation (Jalowiec, Panksepp, Zolovick, Najam, & Herman, 1981). Increased food intake is also observed following morphine injections into the paraventricular nucleus (McLean & Hoebel, 1982; 1983) and the ventromedial hypothalamus (Tepperman, Hirst, & Gowdey, 1981a).

A variety of opiate drugs and endogenous opioid peptides that are putative agonists of different opioid receptor subtypes produce hyperphagia following central or systemic administraion. Beta-endorphin, a putative agonist of the epsilon receptor (Schulz, Wuster, Krenss, & Herz, 1980), increases food intake following injection into either the ventromedial hypothalamus (Grandison & Guidotti, 1977) or the lateral ventricles (McKay, Kenney, Edens, Williams, & Woods, 1981). D-ala-met-enkephalinamide (DALA), a putative delta receptor agonist, increased food intake following paraventricular nucleus injections in rats (McLean & Hoebel, 1982, 1983) and lateral ventricle injections in sheep (Baile et al., 1981), but not following lateral ventricle injections in rats (McKay et al., 1981). Another delta receptor agonist, D-ala-D-leu-enkephalin (DADL) also produces feeding when injected into the ventromedial hypothalamus (Tepperman & Hirst, 1983). Systemic adminstration of EKC and central administration of dynorphin, both kappa receptor agonists

increase food intake in nondeprived rats (Morley & Levine, 1981b; Morley, Levine, Grace, & Kneip, 1982a; Morley, Levine, Grace, & Kneip, 1982b; Sanger & McCarthy, 1981). N-allylnormetazocine (SKF-10,047), a sigma receptor agonist, increased food intake at low doses in free-feeding rats (Gosnell, Levine, & Morley, 1983).

There is also evidence indicating that endogenous opioid peptides are involved in the development of obesity. High levels of beta-endorphin were found in genetically obese mice (ob/ob) and rats (fa/fa) although it is not clear whether beta-endorphin precipitates this condition or is a by-product (Margules, Moisset, Lewis, Shibuya, & Pert, 1978; Rossier, Rogers, Shibasaki, Guillemen, & Bloom, 1979). Short-acting opiate receptor antagonists reduce overeating in genetically obese mice and rats (Margules et al., 1978; McLaughlin & Baile, 1984; Recant, Voyles, Luciano, & Pert, 1980; Shimomura, Oku, Glick, & Bray, 1982; Thornhill, Taylor, Marshall, & Parent, 1982), in rats made obese by hypothalamic lesions (King, Castellanos, Kastin, Berzas, Hauk, Olson, & Olson, 1979) and in rats exposed to dietary regimens that eventually induce obesity (Apfelbaum & Mandenoff, 1981).

The long-term effects of opiate receptor agonists and antagonists on feeding behavior have not been as well documented as short-term acute effects. Brands et al. (1979) found that one injection of naloxone suspended in a slow-release vehicle significantly reduced food intake and body weight in rats over 11 days. Repeated daily injections of naloxone dissolved in saline over 10 days reduced night-time feeding but failed to alter overall food

intake and body weight. On the other hand, repeated daily injections of naloxone in mildly food-deprived rats consistently reduced food intake over a 8 day period (Jalowiec et al., 1981). Daily injections of naltrexone in the squirrel monkey also reduced food intake and body weight with no evidence of tolerance (Herman & Holtzman, 1984). The chronic effect of opiate antagonists on food intake and body weight can be influenced by dietary manipulations. Daily naltrexone injections had a greater inhibitory effect on rats given a sucrose solution in addition to laboratory chow (Marks-Kaufman, Balmagiya, & Gross, 1984). Naltrexone reduced food intake and body weight across a two week injection period in sucrose-fed animals but only reduced food intake but not body weight in the first week of injections in chow-fed animals. Opiate antagonists also have been shown to be more effective in genetically obese rats than their lean littermate controls. Daily administration of nalmefene, a potent antagonist over 21 days reduced food intake and body weight gain to a greater extent in obese rats than in lean rats (McLaughlin & Baile, 1983). The chronic effect of opiate antagonists on intake also appears to show tolerance (Olson, Delatte, Kastin, McLean, Phillpott, & Olson, 1985a) in that rats decreased their intake of a 20% sucrose solution only during the first two of 15 days of naloxone injections. In contrast, chronic injections of opiate receptor agonists do not show tolerance (Jalowiec et al., 1981; Morley et al., 1982b). Morphine consistently increased food intake over a 8 day injection regimen (Jalowiec et al., 1981). This contrasts with the production of tolerance that occurs to morphine's analgesic effects (Martin, Eades,

Thompson, Huppler, & Gilbert, 1976). The kappa receptor agonist, ketocyclazocine injected over a 5 day period also consistently increased food intake with no evidence of tolerance (Morley et al., 1982b).

Rationale

The heterogeneity of opiate receptors has been demonstrated using pharmacological, biochemical and bioassay techniques (e.g., Chavkin & Goldstein, 1981; Lord et al., 1977; Martin et al., 1976). Biochemical studies have also demonstrated the existence of two distinct binding sites for the mu receptor: a high-affinity binding site termed mu-1 and a low-affinity binding site termed mu-2 (Wolozin & Pasternak, 1981). The mu-1 binding site appears to be the common high-affinity binding site for a variety of opiate drugs and opioid peptides (Hazum et al., 1981; Houghten, Johnson, & Pasternak, 1984; Pasternak, 1980; Pasternak et al., 1980a; Zhang & Pasternak, 1981b). The development of irreversible high-affinity opiate receptor antagonists, such as naloxazone and naloxonazine (Hahn & Pasternak, 1982) allowed investigation of the functional significance of the mu-1 binding site in opiate and opioid-mediated actions. Naloxazone and naloxonazine produce long-lasting inhibition of the supraspinal analgesic actions of morphine, metkephamid, ketocyclazocine and DADL; naloxonazine produces these effects at far lower doses (Ling et al., 1983, 1984, 1985; Ling & Pasternak, 1982, 1983; Pasternak, 1980; Pasternak et al., 1980a; Zhang & Pasternak, 1981b). The use of irreversible opiate receptor antagonists has thus far distinguished the mediation of the mu-1 binding site in the opiate modulation of supraspinal analgesia, catalepsy, hypothermia, prolactin release, and acetylcholine turnover, and the apparent non-involvement of the mu-1

binding site in the opiate modulation of spinal analgesia, respiratory depression, physical dependence, sedation, bradycardia, growth hormone release and dopamine turnover (see review: Pasternak & Wood, 1986). The opiate modulation of food intake can also be differentiated as mu-1 mediated and non-mu-1 mediated (Simone et al., 1985). Free-feeding and deprivation-induced feeding were inhibited by pretreatment with either naloxonazine administered 24 h prior to treatment or naloxone administered 15 min prior to treatment, suggesting mu-1 receptor mediation of these effects. On the other hand, glucoprivic feeding induced by administration of 2-deoxy-D-glucose was blocked by naloxone but not by naloxonazine, implying a non-mu-1 receptor mediation. These results indicate that the heterogeneity of opiate receptor mediation of opiate actions extend to feeding behavior.

Administration of different opiate receptor agonists increases food intake with the magnitude and duration of each response dependent upon the agonist employed. For instance, systemic administration of morphine increases food intake in both non-deprived and mildly-deprived (4-5 h) rats with the latter condition producing a more robust feeding response (Jalowiec et al., 1981; Sanger & McCarthy, 1980, 1981). This effect is dose-dependent and reversed by the opiate antagonist, naloxone (Sanger & McCarthy, 1981). Systemic administration of EKC and other benzomorphan derivatives increases food intake in freely-feeding rats in a dose-dependent and naloxone-reversible manner (Lowy & Yim, 1983; Morley et al., 1982b; Sanger & McCarthy, 1981). Increased food intake in freely-feeding rats also

occurs following intracerebroventricular administration of either dynorphin (Morley & Levine, 1981b; Morley et al., 1982a) or DADL (McLean & Hoebel, 1983; Tepperman & Hirst, 1983; Jackson & Sewell, 1985) in dose-dependent and naloxone-reversible manners. Whether these different agonists act to induce feeding through a single, common receptor mechanism is not clear, although specifc receptor subtypes for the mediation of opioid-induced feeding (e.g. kappa: Morley & Levine, 1983; Morley, Levine, Gosnell, & Billington, 1984) have been proposed.

Naloxonazine was used to evaluate whether the mu-1 opioid binding site mediates feeding induced by morphine, EKC, dynorphin and DADL by determining whether pretreatment with this antagonist 24 h prior to each opiate treatment would block the subsequent hyperphagic responses. The presence or absence of effects upon food intake would be interesting in light of the ability of the mu-1 antagonists naloxazone and naloxonazine a) to inhibit free-feeding and deprivation-induced feeding, and b) to block supraspinal analgesia induced by morphine, ketocyclazocine and DADL (Ling et al., 1983, 1984, 1985; Pasternak, 1980; Pasternak et al., 1980a; Zhang & Pasternak, 1981b). The effects of naloxonazine upon agonist-induced food intake was compared with the known antagonistic effects of naloxone pretreatment (5 min) upon hyperphagia induced by morphine (e.g. Sanger & McCarthy, 1981), EKC (Lowy & Yim, 1983), dynorphin (Morley & Levine, 1981b; Morley et al., 1982a) and DADL (Mclean & Hoebel, 1983; Tepperman & Hirst, 1983). If both naloxone and naloxonazine inhibit any of the forms of opioid-induced feeding it would suggest that the mu-1

binding site is involved in each particular system. In contrast, failure of naloxonazine to affect any of the forms of opioid-induced feeding would imply a non-mu-1 receptor mechanism in these feeding models and dissociate opiate ingestive effects from opiate analgesic effects.

The second set of experiments compared the effects of chronic administration of naloxone and naloxonazine to pinpoint possible mu-1 involvement in food intake and body weight maintenance. As mentioned previously, repeated administration of opiate receptor antagonists has yielded equivocal results. While several reports indicate that chronic naloxone or naltrexone treatment decreased food intake and body weight in rats (Brands et al., 1979; Jalowiec et al., 1981; Marks-Kaufman et al., 1984) and prevented the occurrance of cafeteria-induced obesity (Apfelbaum & Mandenoff, 1981), other studies indicate that these chronic suppressive effects were transitory and resulted in a form of tolerance (Brands et al., 1979; Olson et al., 1985a). The second series of experiments examined the role of the mu-1 binding site in the maintenance of food intake and body weight by comparing the effects of daily intravenous administration of naloxone and naloxonazine in three different types of feeding models. Full-grown adult rats were used initially to determine the effects of daily administration of naloxone and naloxonazine on body weight and food intake. Second, adolescent, but sexually-mature rats were to determine antagonist effects upon intake and weight during a phase of dynamic growth. Third, in an effort to evaluate chronic antagonists effects upon obesity, the effects of naloxonazine and

naloxone were evaluated in adult rats made obese by a 'cafeteria' diet and maintained on that diet during testing.

General Methods

Subjects: Albino Sprague Dawley male rats (250–600g) were used in all experiments. The animals were housed individually in wire mesh cages on a 12 h light (0800): 12h dark (2000) cycle at ambient temperatures between 21 and 25°C. Purina Rodent Chow and tap water were available ad libitum except where specifically stated in the protocol.

Lateral Ventricle Cannulations: Animals in Experiments 1c and 1d received chlorpromazine HCl (3 mg/ml normal saline/kg body weight, i.p.) 20 min before anesthetization with ketamine HCl (100 mg/ml normal saline/kg body weight, i.m.). One stainless steel 22 gauge guide cannula (Plastic Products) was stereotaxically (Kopf) aimed so that the tip was positioned 0.3 mm above the left lateral ventricle. With the incisor bar set at +5 mm, the coordinates were 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 three stainless steel anchor screws with dental acrylic. All animals were allowed 10 days to recover from surgery before testing began. Intracerebroventricular infusions were made at a rate of 1 ul every 20 sec through a stainless steel 28 gauge internal cannula (Plastic Products) which protruded 0.5 mm past the tip of the guide cannula.

Histology: Following experimental testing, all cannulated rats were

anesthetized with sodium pentobarbital (100 mg/2ml normal saline/kg body weight, i.p.) and perfused through transcardiac puncture with 0.9% saline followed by 10% buffered formalin. Each brain was removed, blocked, sliced into 40 um sections, mounted and stained with cresyl violet for cell body visualization. Coronal sections through the lateral ventricle were analyzed for cannula placement. Only animals with cannula tips located in the lateral ventricle were included for data analysis.

Jugular Catheterizations: This procedure was used in all experiments and was a modification of a procedure initially developed by Weeks (1962). After anesthetization with ketamine HCl (100 mg/ml normal saline/kg body weight, i.m.), a longitudinal incision was made over the right external jugular vein. The vein was then exposed (5-10 mm) by blunt dissection and connective tissue was removed. A stainless steel spatula elevated the vein which was punctured with a 19 gauge needle. Vinyl intravenous tubing (BOLAB V-3, 20 mm) was then inserted and subsequently anchored to the vein with 4-0 suture and cyanoacrylate glue by a cuff (BOLAB V-5). A loop was made in the remaining tubing which was placed in a pocket created by hemostatic forceps left of midline. The distal end of the tubing was passed through the skin subcutaneously to an incision previously made on the dorsal surface of the neck. The tubing was anchored there with 2-0 suture and cyanoacrylate glue by a second cuff (V-5). The catheter extended an inch past the skin and was filled with heparin (50 U/ml normal saline) to prevent blood clots within the tubing. The end of

the tubing was closed off by either a knot made in the tubing or by a crimped 22 gauge needle depending upon whether the experiment required one injection or multiple injections. When the animal received an intravenous injection, heparin (0.2 ml) was administered initially to clear the line and subsequently to prevent further clotting.

Data Analysis: Split plot analyses of variance were performed to determine the presence of significant effects or interactions. Where appropriate, Dunnett comparisons were used to determine experimental differences from control means. Dunn comparisons were used to determine differences among experimental groups. Correlation coefficients were used to evaluate changes in body weight and food intake for the chronic opiate antagonist studies (Kirk, 1968).

Experiment 1: Naloxonazine and Naloxone Effects upon Opioid Agonist Feeding.

Experiment 1a: Morphine Protocol

Fourteen catheterized rats (450-600 g) received the following four injection conditions: a) vehicle (1 ml normal saline/kg body weight, s.c.), b) morphine sulfate (Pennick Laboratories, 5 mg/ml normal saline/kg body weight, s.c.), c) naloxone (Endo Laboratories; 10 mg/ml normal saline/kg body weight, s.c.) paired with morphine, and d) naloxonazine (10 mg/kg, i.v. administered in 5 mg/ml normal saline/0.2% glacial acetic acid) paired with morphine. Naloxonazine was provided by Dr. Pasternak and was administered 24 h prior to morphine treatment to allow for the development of irreversible binding to the mu-1 binding site (Hahn, Carroll-Buatti, & Pasternak, 1982; Johnson & Pasternak, 1984; Ling, Simantov, Clark, & Pasternak, 1986). Naloxonazine will reversibly bind to many classes of opioid binding sites on a short-term basis (Ling et al., 1986). The 10 mg/kg dose of naloxonazine was chosen because: a) higher doses have similar effects on feeding (Simone et al., 1985), b) this dose produces maximal inhibition of mu-1 sites and morphine analgesia (Ling et al., 1986). The 10 mg/kg dose of naloxonazine dose-dependently blocks morphine analgesia by decreasing its peak effects and duration of action. In addition, higher doses of naloxonazine antagonize other opioid binding sites besides mu-1 (Ling et al., 1986). The doses and time interval (5 min) of naloxone and morphine

treatments were chosen on the basis of previous reports (Jalowiec et al., 1981; Sanger & McCarthy, 1980). Rats were mildly food and water deprived for 5 h before and for 0.5 h following morphine or vehicle administration, a condition which elicits reliable feeding responses for morphine (Jalowiec et al., 1981). Food and water were returned to the cages and food intake was measured over 4 h after adjustments for spillage which was measured by placing paper towels under each cage and collecting and weighing food particles. All injection conditions occurred 1-2 h into the light cycle and were separated by a minimum of 3 days.

In the above protocol naloxone and naloxonazine were administered by different routes and at different time intervals. Therefore, to insure that any differences between naloxone and naloxonazine reflected their receptor sensitivities and not those other variables, an additional group of eight rats, matched for food intake following vehicle treatment, were exposed to the following injection conditions: a) vehicle, b) morphine (5 mg/kg, s.c.), c) naloxone (10 mg/kg, i.v.) administered 24 h prior to morphine, and d) naloxone administered 5 min before morphine. Identical deprivation and intake conditions were employed.

Experiment 1b: EKC Protocol

An additional group of 13 catheterized rats received: a) vehicle, b) EKC (NIDA; 5 mg/ml normal saline/kg body weight, s.c.), c) naloxone (10 mg/kg, s.c.) paired with EKC, and d) naloxonazine (10 mg/kg, i.v.) paired with EKC. Again, a minimum of 3 days elapsed

between conditions. Since previous reports indicated that EKC elicits a reliable feeding response in sated rats, injections were administered to freely-feeding rats 1-2 h into the light cycle (Morley et al., 1982b; Sanger & McCarthy, 1981). Food intake was assessed 4 h later. Results of this experiment showed that naloxone at 10 mg/kg did not block EKC hyperphagia at 5 mg/kg therefore an additional group of 19 animals received identical treatments except that the EKC dose was 2 mg/kg.

Experiment 1c: Dynorphin Protocol

An additional group of 12 cannulated and catheterized rats received at 3 day intervals: a) vehicle, b) dynorphin (Dynorphin A 1-13, Peninsula Laboratories; 10 ug/5 ul normal saline, i.c.v), c) naloxone (10 mg/kg, s.c.) paired with dynorphin, and d) naloxonazine (10 mg/kg, i.v.) paired with dynorphin. The dynorphin dose was chosen because previous reports indicated a reliable feeding response in freely-feeding rats (Morley & Levine, 1981b). Injections were made 1 to 2 h into the light cycle and intake was assessed 1 h later (Morley & Levine, 1981b). The shorter feeding interval was based upon previous reports and due to the more rapid degradation properties of the endogenous opioid.

Experiment 1d: DADL Protocol

An additional group of 19 cannulated and catheterized rats received at 3 day intervals: a) vehicle, b) DADL (Peninsula Labora-

tories; 10 ug/5 ul normal saline, i.c.v), c) naloxone (10 mg/kg, s.c.) paired with DADL, and d) naloxonazine (10 mg/kg, i.v.) paired with DADL. This DADL dose increases feeding in freely-feeding rats (Tepperman & Hirst, 1983). Again, injections were made 1-2 h into the light cycle to freely-feeding rats and intake was assessed 1 h later.

Results

Morphine Hyperphagia: Morphine elicited a significant 25% increase in food intake as compared to vehicle treatment (Table 1). When the rats were pretreated with naloxone 5 min before morphine, food intake failed to differ (21% decrease) from vehicle treatment, but was significantly decreased by 37% relative to morphine treatment alone, indicating that naloxone completely eliminated morphine-induced hyperphagia. In contrast, when the rats were pretreated with naloxonazine 24 h before morphine, food intake was significantly increased relative to either vehicle treatment (56% increase) or morphine treatment alone (24% increase), indicating a clear dissociation between naloxone and naloxonazine pretreatments.

Table 2 indicates that these dissociations between naloxone and naloxonazine pretreatment upon morphine-induced hyperphagia were not due to differences in either the route of administration (s.c. vs i.v.) or the time interval (5 min vs 24 h). While morphine alone significantly increased intake by 32% relative to vehicle treatment, intravenous pretreatment with naloxone 5 min prior to morphine induced food intake which failed to differ from vehicle treatment (27% decrease), but which was significantly reduced (45% decrease) relative to morphine treatment alone. Thus, both intravenous and subcutaneous naloxone pretreatment 5 min prior to morphine eliminated morphine hyperphagia, an effect expected of its short duration of action. In contrast, intravenous pretreatment with naloxone 24 h prior to morphine failed to affect the latter's hyperphagic proper-

TABLE 1. Differential alterations in morphine-induced increases in food intake by naloxone and naloxonazine pretreatment.*

Condition	Food Intake (g±SEM)
Vehicle	6.35 (0.60)
Morphine (5 mg/kg, s.c.)	7.96 (0.76)*
Naloxone (10 mg/kg, s.c.)/Morphine (5 mg/kg, s.c.)	5.03 (0.52)+
Naloxonazine (10 mg/kg, i.v.)/Morphine (5 mg/kg, s.c.)	9.90 (0.61)*+

* Significant differences were observed among the four conditions ($F(3,38)=13.92$, $P<.001$).

* Significantly greater than vehicle (Dunnett comparison, $P<.05$)

+ Significantly different from morphine alone (Dunn comparisons, $P<.05$).

TABLE 2. Ability of short-term, but not long-term naloxone pretreatment to alter morphine-induced increases in food intake.*

Condition	Food Intake (g±SEM)
Vehicle	4.16 (0.93)
Morphine (5 mg/kg, s.c.)	5.53 (0.73)*
Naloxone (10 mg/kg, i.v.)-5 min-Morphine	3.04 (0.28)+
Naloxone (10 mg/kg, i.v.)-24 h-Morphine	5.84 (0.62)*

* Significant differences were observed among the four conditions ($F(3,21)=4.91$, $P<.01$).

* Significantly greater than vehicle (Dunnett comparison, $P<.05$)

+ Significantly different from morphine alone (Dunn comparisons, $P<.05$).

ties (40% increase relative to vehicle, 6% increase relative to morphine alone).

EKC Hyperphagia: EKC significantly increased food intake in freely-feeding rats over vehicle levels following administration of the 2 mg/kg (51% increase) and 5 mg/kg (47% increase) doses (Table 3). Following naloxone pretreatment, the 2 mg/kg dose of EKC induced food intake which failed to differ from vehicle values (3% increase) but was significantly reduced (32%) relative to EKC alone. This pattern was altered markedly by increasing the EKC dose. Naloxone pretreatment 5 min prior to a 5 mg/kg dose of EKC elicited food intake which failed to differ (5% decrease) from EKC alone, but which was significantly increased by 40% above vehicle values. Thus, naloxone reversal of EKC hyperphagia was dependent upon the EKC dose employed. Pairing of naloxonazine and EKC produced a different pattern in that this condition elicited food intake which failed to differ from EKC alone (2 mg/kg: 3% decrease; 5 mg/kg: 13% increase) and which was significantly increased over vehicle values at both the 2 mg/kg (47% increase) and the 5 mg/kg (66% increase) EKC doses. Hence while naloxone could eliminate EKC hyperphagia at the lower, but not the higher EKC doses, naloxonazine failed to affect either condition.

Dynorphin Hyperphagia: Dynorphin significantly increased food intake by 122% in freely-feeding rats relative to vehicle values (Table 4). Food intake following naloxone paired with dynorphin failed to differ

TABLE 3. Differential alterations in EKC-induced increases in food intake by naloxone and naloxonazine pretreatment.*

Condition	Food Intake (g; SEM)	
	EKC (2 mg/kg)	EKC (5 mg/kg)
Vehicle	3.85 (0.43)	3.64 (0.37)
EKC	5.82 (0.63)*	5.35 (0.50)*
Naloxone (10 mg/kg)/EKC	3.98 (0.50)+	5.08 (0.79)*
Naloxonazine (10 mg/kg)/EKC	5.65 (0.53)*	6.03 (0.67)*

* Significant differences were observed among the four conditions in both the 2 mg/kg ($F(3,36)=4.47$, $P<.001$) and the 5 mg/kg ($F(3,54)=6.26$, $P<.001$) groups.

* Significantly greater than vehicle (Dunnett comparison, $P<.05$)

+ Significantly less than EKC alone (Dunn comparison, $P<.05$).

TABLE 4. Differential alterations in dynorphin-induced increases in food intake by naloxone and naloxonazine pretreatment.*

Condition	Food Intake (g±SEM)
Vehicle	0.65 (0.27)
Dynorphin (10 ug, i.c.v.)	1.44 (0.42)*
Naloxone (10 mg/kg, s.c.)/Dynorphin (10 ug, i.c.v.)	0.28 (0.17)+
Naloxonazine (10 mg/kg, i.v.)/Dynorphin (10 ug, i.c.v.)	1.36 (0.35)*

* Significant differences were observed among the four conditions ($F(3,33)=3.46$, $<.027$).

* Significantly greater than vehicle (Dunnett comparison, $P<.05$)

+ Significantly less than dynorphin alone (Dunn comparisons, $P<.05$).

from vehicle levels but was significantly lower than dynorphin alone. In contrast, food intake following naloxonazine paired with dynorphin failed to differ from dynorphin alone, and was significantly increased relative to vehicle values.

DADL Hyperphagia: DADL administration resulted in a significant 236% increase in food intake in freely-feeding rats relative to vehicle values (Table 5). Food intake following naloxone paired with DADL failed to differ from vehicle levels but was significantly lower than DADL alone. In contrast, food intake following naloxonazine paired with DADL failed to differ from DADL alone, and was significantly increased relative to vehicle values.

Table 6 is a compilation of the food intake under all drug conditions included for the sake of clarity. Since there were conditions of food deprivation and free-feeding it is difficult to compare across drug groups yet it is evident that while naloxone blocked opiate-induced feeding in the morphine, EKC (2 mg/kg), dynorphin and DADL conditions, naloxonazine had no effect in any of the groups.

TABLE 5. Differential alterations in DADL-induced increases in food intake by naloxone and naloxonazine pretreatment.*

Condition	Food Intake (g; SEM)
Vehicle	0.53 (0.19)
DADL (10 ug, i.c.v.)	1.79 (0.35)*
Naloxone (10 mg/kg, s.c.)/DADL (10 ug, i.c.v.)	0.65 (0.08)+
Naloxonazine (10 mg/kg, i.v.)/DADL (10 ug, i.c.v.)	2.05 (0.31)*

* Significant differences were observed among the four conditions ($F(3,54)=10.23$, $P<.0001$).

* Significantly greater than vehicle (Dunnett comparison, $P<.05$)

+ Significantly less than DADL alone (Dunn comparisons, $P<.05$).

TABLE 6. Comparison of the alterations in morphine, EKC, dynorphin and DADL-induced increases in food intake by naloxone and naloxonazine pretreatment.

Condition	Food Intake (g; SEM)				
	Morphine*	EKC (2)+	EKC (5)+	Dynorphin++	DADL++
Vehicle	6.35	3.85	3.64	0.65	0.53
Drug alone	7.96	5.82	5.35	1.44	1.79
Drug plus naloxone	5.03	3.98	5.08	0.28	0.65
Drug plus naloxonazine	9.90	5.65	6.03	1.36	2.05

* Based on 4 h intake after mild food deprivation.

\+ Based on 4 h intake in freely-feeding rats.

++ Based on 1 h intake in freely-feeding rats.

Experiment 2: Chronic Opiate Antagonist Effects Upon Body Weight Maintenance and Food Intake.

Experiment 2a: Adult Protocol

Twenty-seven adult rats (450-650g) received external jugular catheters and were allowed two days following recovery from surgery. The rats were divided into three matched groups based upon their body weights following three days of vehicle injections (2 ml normal saline/kg body weight, i.v.) 4-5 h into the light cycle. Following establishment of these baseline conditions, single, daily intravenous injections of either vehicle (Group 1, n=11), naloxone (10 mg/2 ml normal saline/kg body weight, Endo Laboratories; Group 2, n=8) or naloxonazine (10 mg/2 ml normal saline with 0.2% acetic acid, Group 3, n=8) were administered daily over 14 days at 4-5 h into the light cycle. Body weight and food intake were recorded every day for each animal including adjustments for spillage. Any animals in the experimental groups that developed catheter leaks were excluded from data analysis; this accounts for the unequal samples in this and subsequent groups. After the last injection, recovery body weight was recorded for each animal at weekly intervals for three weeks.

Experiment 2b: Adolescent Protocol

Adolescent though sexually-mature rats (60 days of age, 250-425g) were employed to assess naloxone and naloxonazine effects on body weight gain and food intake in rats that were experiencing

dynamic growth. Each rat received external jugular catheters and were allowed two days to recover from surgergy. Each rat received three days of vehicle injections (2 ml normal saline/kg body weight, i.v.) 4-5 h into the light cycle, and were randomly assigned to three groups based on body weight. Single, daily intravenous injections of either vehicle (Group 1, n=13), naloxone (Group 2, n=5; 10 mg/2 ml normal saline/kg body weight) or naloxonazine (Group 3, n=7; 10 mg/2 ml normal saline with 0.2% acetic acid) were administered daily over 14 days. Body weight and food intake were recorded every day including adjustments for spillage. Recovery of body weight was recorded for each animal for three weeks after the last injection.

Experiment 2c: Dietary Obesity Protocol

Adult rats (90 days, 275-490g) were exposed to a cafeteria diet to evaluate naloxone and naloxonazine effects on dietary obesity. Rats matched for body weight were maintained on either a control diet (Purina Rodent Chow and tap water ad libitum; n=6) or a "cafeteria" diet (n=27). The cafeteria diet consisted of laboratory chow (3.6 kcal/g: 25.7% protein, 11.7% fat, 62.6% carbohydrates), fat (67% ground lab chow plus 33% vegetable shortening; 5.5 kcal/g: 11.3% protein, 61.3% fat, 27.5% carbohydrates), milk solution (41% evaporated milk plus 59% tap water plus 19% sugar; 1.3 kcal/g: 9.1% protein, 23.4% fat, 67.4% carbohydrates), chocolate-chip cookies (Sunshine; 4.8 kcal/g: 4.8% protein, 40.4% fat, 54.6% carbohydrates) and tap water ad libitum (Sclafani, Aravich, & Landman, 1981). All constituents were replaced fresh daily. All rats were weighed

weekly, until the cafeteria rats (mean = 607g) weighed an average of 100 g more than the control rats (mean = 506g) after six weeks. When this criterion was reached, all rats received external jugular catheters. After two days of surgical recovery, all rats received daily injections of saline vehicle for three days. The cafeteria-treated animals were then divided into three groups: vehicle (n=13), naloxone (n=9), or naloxonazine (n=5). The control group and three cafeteria groups received their respective injections daily over a 8 day period while being maintained on their respective diets. Body weight of all rats as well as sweetened milk consumption of the cafeteria rats were recorded daily.

Results

Adult Protocol: Body weight in adult rats changed significantly across the injection time course ($F(16,384)=11.26$, $P<.001$) (Figure 1). There was also an interaction between groups and the time course ($F(32,384)=2.96$, $P<.001$). Rats treated with vehicle injections failed to display significant changes in body weight across the injection time course relative to their baseline values. In contrast, rats treated with either naloxone or naloxonazine displayed significant reductions in body weight over the injection time course relative to both rats treated with vehicle and their respective baseline values (Dunnett comparison, $P<.05$). A significant loss of body weight was observed in both naloxone-treated and naloxonazine-treated animals by the second day of experimental treatment and persisted across the entire experimental time course, except on the fifth day of treatment in the naloxone-treated group. The weight loss in both experimental groups appeared to be gradual and parallel. By the end of experimental treatment naloxone significantly reduced body weight by 28 g or 5% while naloxonazine reduced body weight by 36 g or 7%. Naloxonazine's effect upon adult body weight was significantly greater than that of naloxone only on the tenth day of experimental treatment (Dunn comparisons, $P<.05$). The losses in body weight were recovered to vehicle values within three weeks after cessation of antagonist treatments. Figure 2 portrays body weights

Figure 1. Alterations in body weight (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 14-day injection regimen in adult rats (Bl - baseline; + significantly less than vehicle (Dunnett comparison, P<.05); * significantly less than naloxone (Dunn comparison, P<.05)).

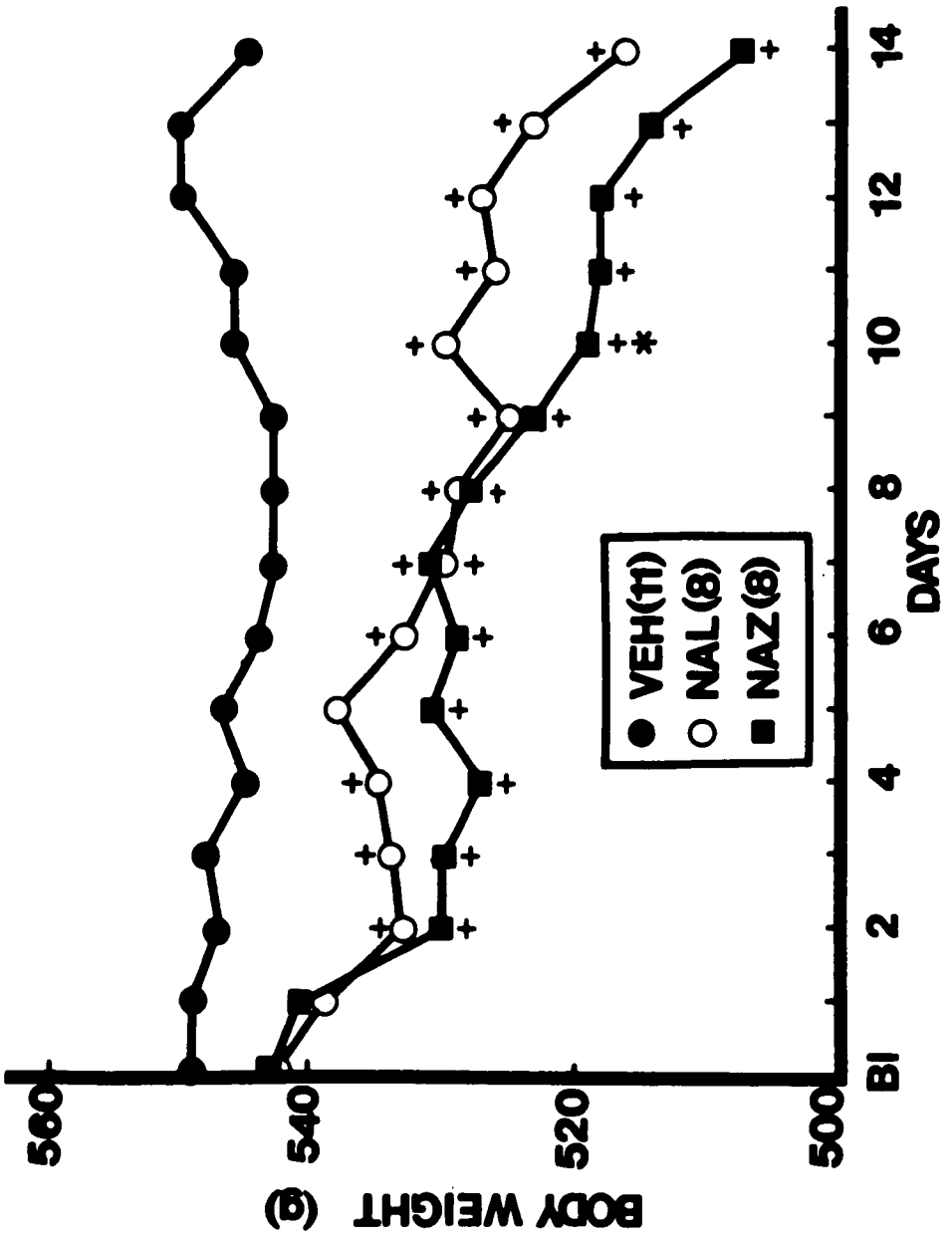
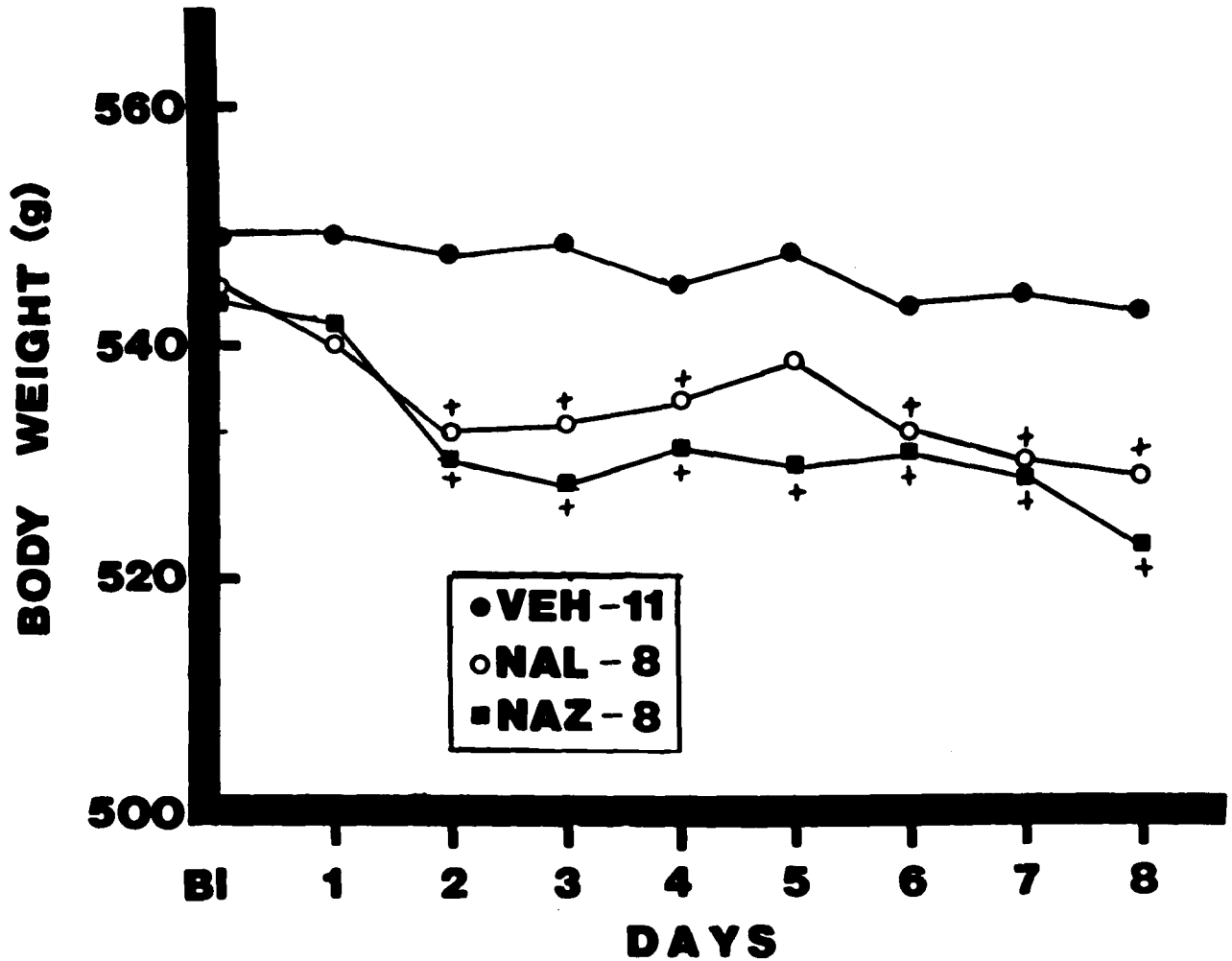

BODY WEIGHT (g)
560
540
520
500
BI
2
4
6
8
10
12
14
DAYS
VEH(11)
NAL(8)
NAZ(8)
*

Figure 2. Alterations in body weight (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 8-day injection regimen in adult rats (Bl - baseline; + significantly less than vehicle (Dunnett comparison, P<.05); * significantly less than naloxone (Dunn comparison, P<.05)).


BODY WEIGHT (g)
560
540
520
500
BI
1
2
3
4
5
6
7
8
DAYS
●VEH-11
○NAL-8
■NAZ-8

for adult animals after eight days of treatment for the sake of clarity and in order to be able to compare it more easily with body weights of dietary obese animals which received treatment for only 8 days (see Figure 9). Since the baseline body weights of the adult animals were slightly, though not statistically different, difference scores were calculated. Figure 3 depicts body weight difference scores of the adult animals. The difference score is the experimental day body weight minus the baseline body weight. Body weight difference scores was signficantly reduced among groups ($F(2,24)=4.89$), $P<.01$), across the injection time course ($F(13,312)=6.18$, $P<.001$) and for the interaction between groups and the time course ($F(26,312)=2.55$, $P<.001$).

Food intake was significantly reduced among groups ($F(2,24)=7.41$, $P<.003$) and there was a significant interaction between groups and the injection time course ($F(32,384)=1.81$, $P<.005$) (Figure 4). Rats treated with vehicle injections failed to display significant changes in food intake across the injection time course relative to their baseline values. In contrast, both naloxone-treated and naloxonazine-treated rats displayed significant reductions in food intake over the injection time course relative to both vehicle-treated rats and their respective baseline values (Dunnett comparisons, $P<.05$). Naloxone treatment significantly reduced intake after the second, tenth, eleventh and fourteenth injections. Naloxonazine treatment significantly reduced intake after the

Figure 3. Body weight difference scores (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 14-day injection regimen in adult rats (B1 - baseline; + significantly less than vehicle (Dunnett comparison, P<.05)

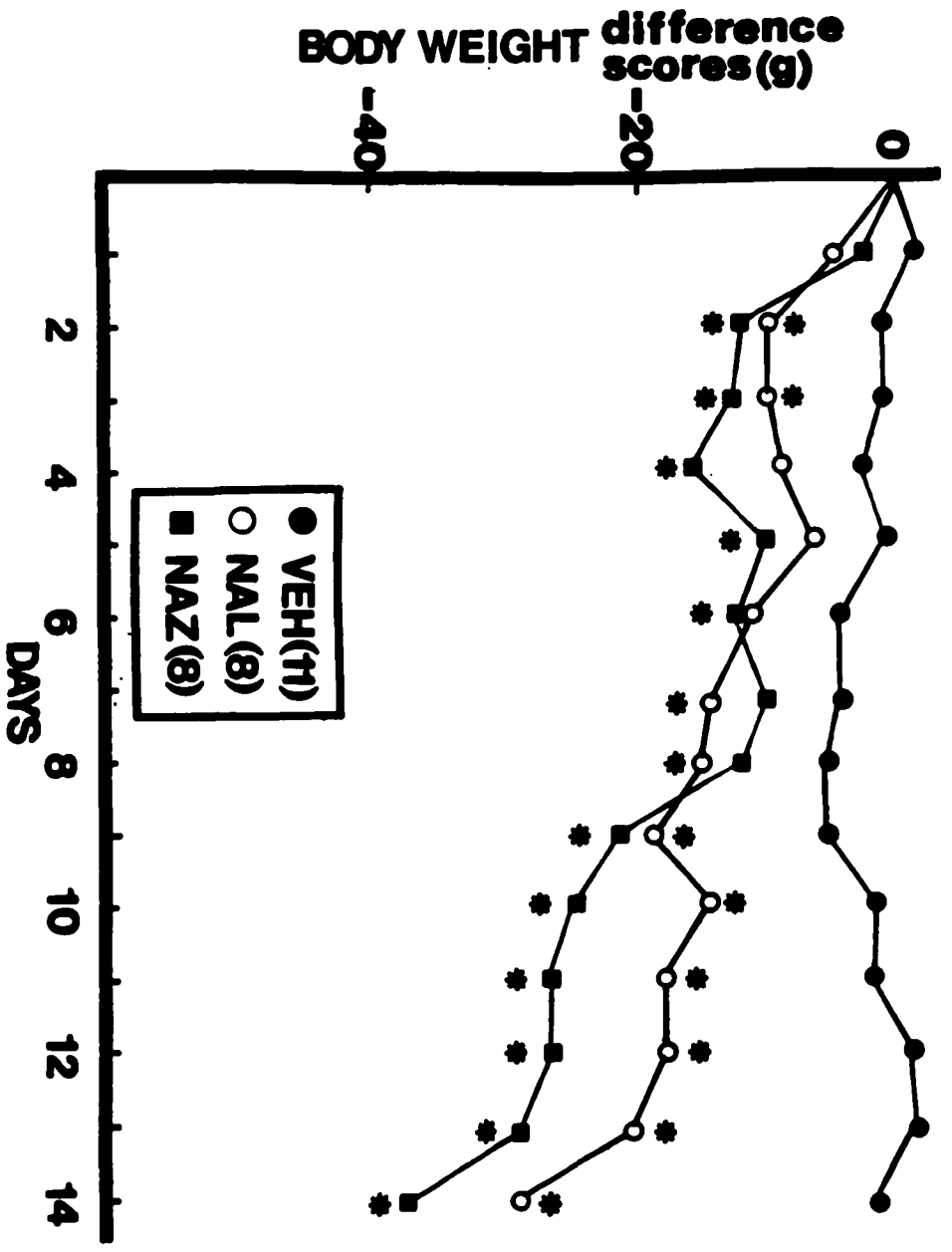
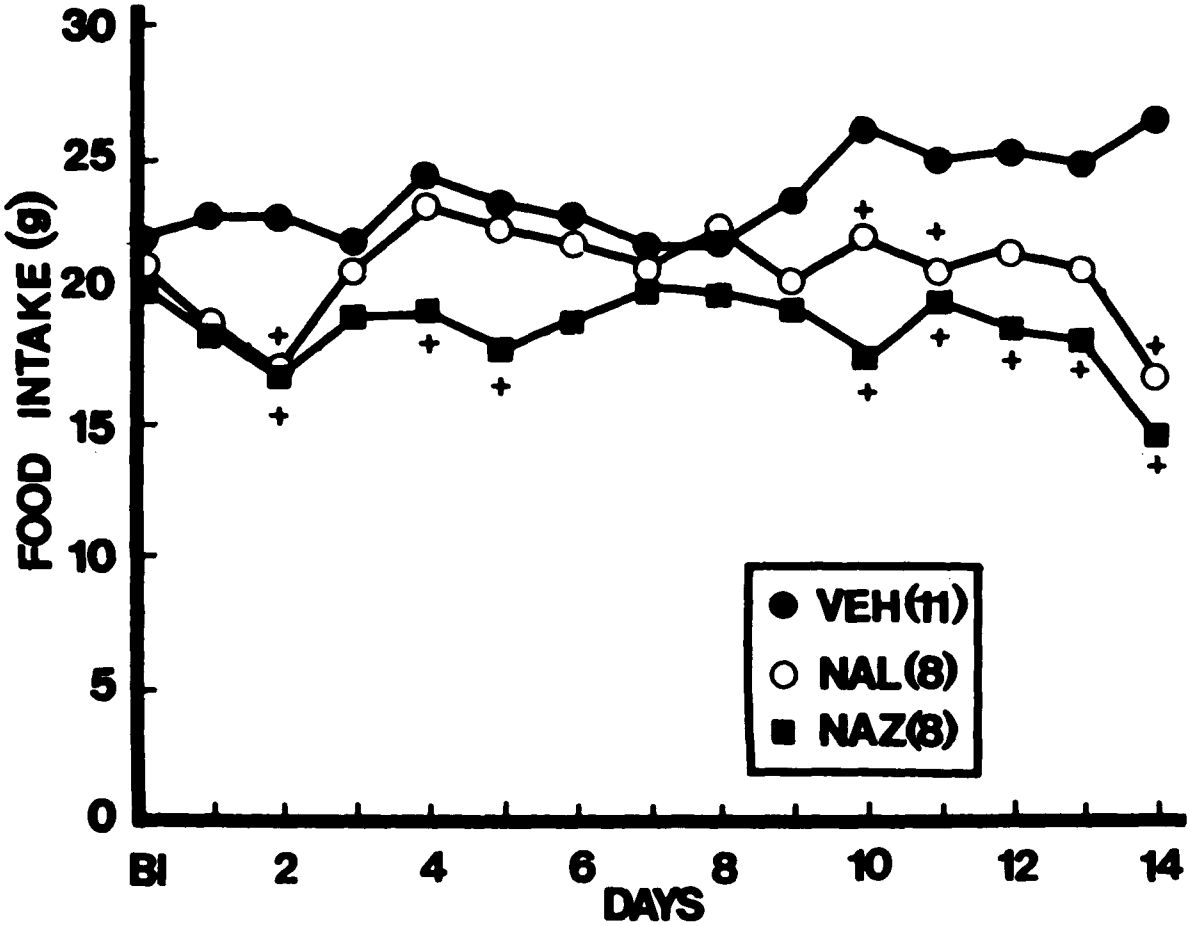

BODY WEIGHT difference scores (g)
-40
-20
0
2
4
6
8
10
12
14
DAYS
● VEH(11)
○ NAL(8)
■ NAZ(8)

Figure 4. Alterations in food intake (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 14-day injection regimen in adult rats (Bl - baseline; + significantly less than vehicle (Dunnett comparison, P<.05)).

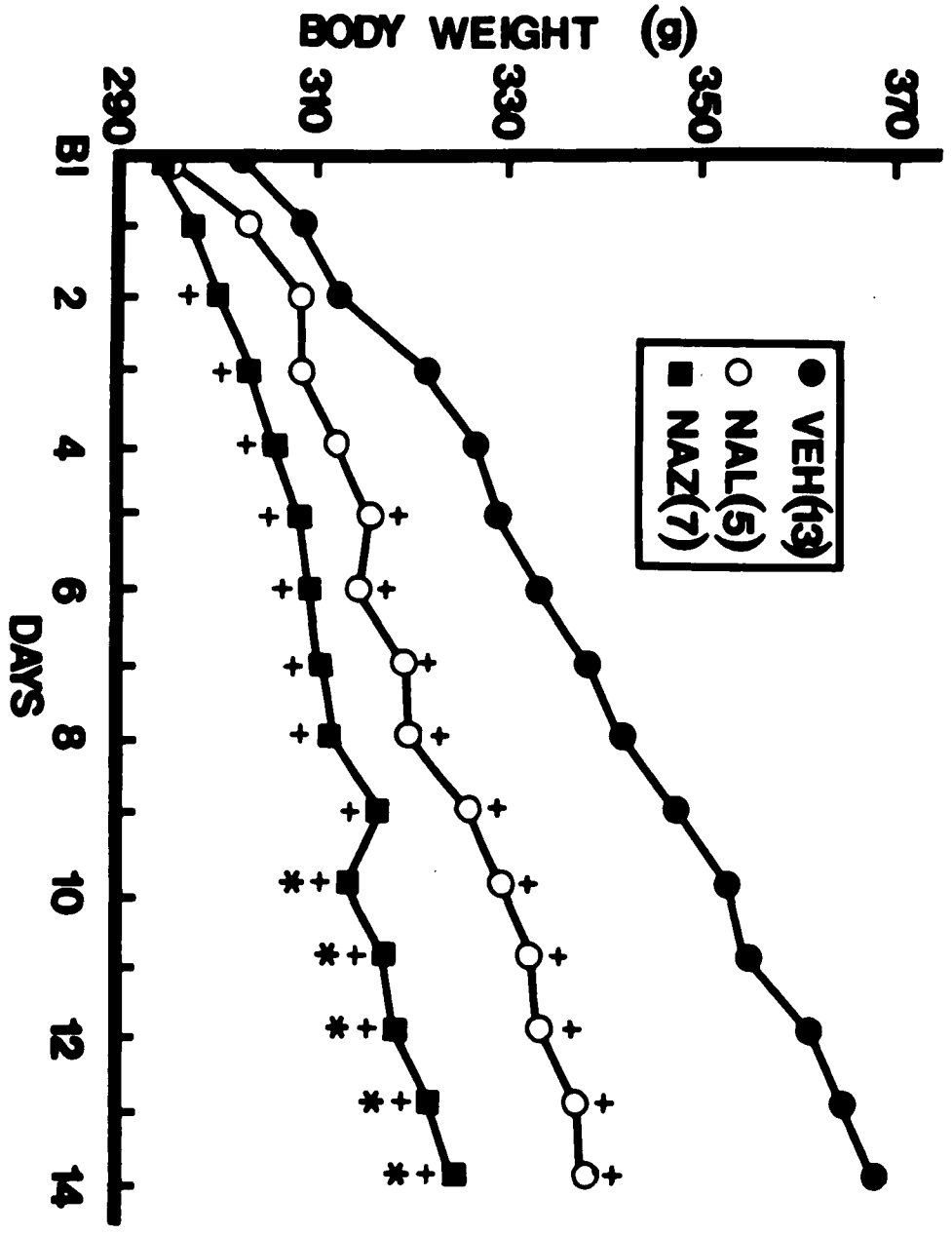

30
25
20
15
10
5
0
FOOD INTAKE (g)
BI
2
4
6
8
10
12
14
DAYS
VEH(11)
NAL(8)
NAZ(8)

second, fourth, and fifth injections as well as the tenth through fourteenth injections. Over the experimental time course, naloxone treatment signficantly reduced food intake by 4% (0.7 g/day) relative to baseline values and by 13% (3 g/day) relative to corresponding vehicle values. Naloxonazine treatment significantly reduced food intake by 10% (2g/day) relative to baseline values, and by 22% (5.3 g/day) relative to corresponding vehicle values. Although naloxonazine appeared to produce greater reductions in food intake than naloxone, the intake of each group failed to differ significantly from each other over the experimental time course (Dunn comparisons, $P<.05$).

The relationship between drug-induced changes in body weight and drug-induced changes in food intake was then assessed by calculating correlation co-efficients. As expected, body weight and food intake changes were not related in vehicle-treated rats ($r(12)=.001$). However, weight loss and food intake reductions were significantly related in naloxone-treated rats ($r(12)=.749$, $P<.01$) and accounted for 56% of the variance. Weight loss and food intake reductions approached significance in naloxonazine-treated rats ($r(12)=.523$, $P<.06$), and accounted for 23% of the variance.

Adolescent Protocol: Figure 5 illustrates the significant retardation of body weight gain in adolescent rats observed among groups ($F(2,22)=4.18$, $P<.029$), across the injection time course ($F(14, 308)=43.26$, $P<.001$) and for the interaction between groups and

Figure 5. Alterations in body weight (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 14-day injection regimen in adolescent rats (Bl - baseline; + significantly less than vehicle (Dunnett comparison, P<.05); * significantly less than naloxone (Dunn comparison, P<.05)).


BODY WEIGHT (g)
290
310
330
350
370
BI
2
4
6
8
10
12
14
DAYS
VEH(13)
NAL(5)
NAZ(7)

the time course ($F(28,308)=3.55, P<.001$). Vehicle-treated adolescent rats displayed the expected significant increases in body weight relative to their baseline values across the injection time course with significant effects emerging after the third injection day (Dunnett comparisons, $P<.05$). Naloxone-treated rats also displayed significant increases in body weight relative to their baseline values across the injection time course, but significant effects only emerged after the fifth day of experiment treatment. Naloxonazine-treated rats displayed significant increases in body weight relative to their baseline values across the injection time course with significant effects emerging on the second day. The weight gain observed in naloxone-treated and naloxonazine-treated rats across the regimen differed from the weight gain observed with vehicle-treated rats. Body weight gain was significantly retarded relative to vehicle treatment in naloxone-treated rats after the third injection day and persisted across the time course of treatment. Weight gain in naloxonazine-treated rats was significantly retarded after the second injection and persisted across the time course (Dunnett comparisons, $P<.05$). The weight gain for vehicle-treated rats totalled 66 g (4.7 g/day gain) over the 14-day regimen. Naloxone significantly suppressed body weight gain to 44 g (3.1 g/day gain) over the 14-day regimen, a 33% reduction relative to vehicle treatment. Naloxonazine significantly suppressed body weight gain to 31 g (2.2 g/day gain) over the 14-day regimen, a 53% reduction relative to vehicle treatment. The retardation in body weight gain weight gain was significantly more pronounced in naloxonazine-treated

rats relative to naloxone-treated rats after the tenth injection (Dunn comparisons, $P<.05$). The retardations in body weight gain produced by both antagonists were recovered to vehicle values within three weeks of antagonist treatment. Figure 6 portrays body weights for adolescent animals after eight days of treatment, again, for the sake of clarity and in order to be able to compare it more easily with body weights of dietary obese animals which received treatment for only 8 days (see Figure 9). The baseline body weights of the adolescent animals were also slightly, though not statistically different, so difference scores were calculated. Figure 7 depicts body weight difference scores of the adolescent animals. Difference scores were significantly different among groups ($F(2,22)=7.82$, $P<.005$), across the injection time course ($F(13,286)=39.64$, $P<.001$) and for the interaction between groups and the time course ($F(26,286)=2.32$, $P<.001$).

Figure 8 illustrates the significant reduction in food intake observed among groups ($F(2,22)=9.80$, $P<.001$) and for the interaction between groups and the injection time course ($F(28,308)=1.58$, $P<.035$). Vehicle-treated rats failed to display significant changes in food intake over the 14-day regimen, but increased intake by 14% (3.6 g/day) relative to baseline values. This parallels the increased weight gain exhibited by these vehicle-treated rats. In contrast, naloxone-treated rats exhibited significant decreases in food intake relative to vehicle-treated rats after the sixth through

Figure 6. Alterations in body weight (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 8-day injection regimen in adolescent rats (Bl - baseline; + significantly less than vehicle (Dunnett comparison, P<.05); * significantly less than naloxone (Dunn comparison, P<.05)).

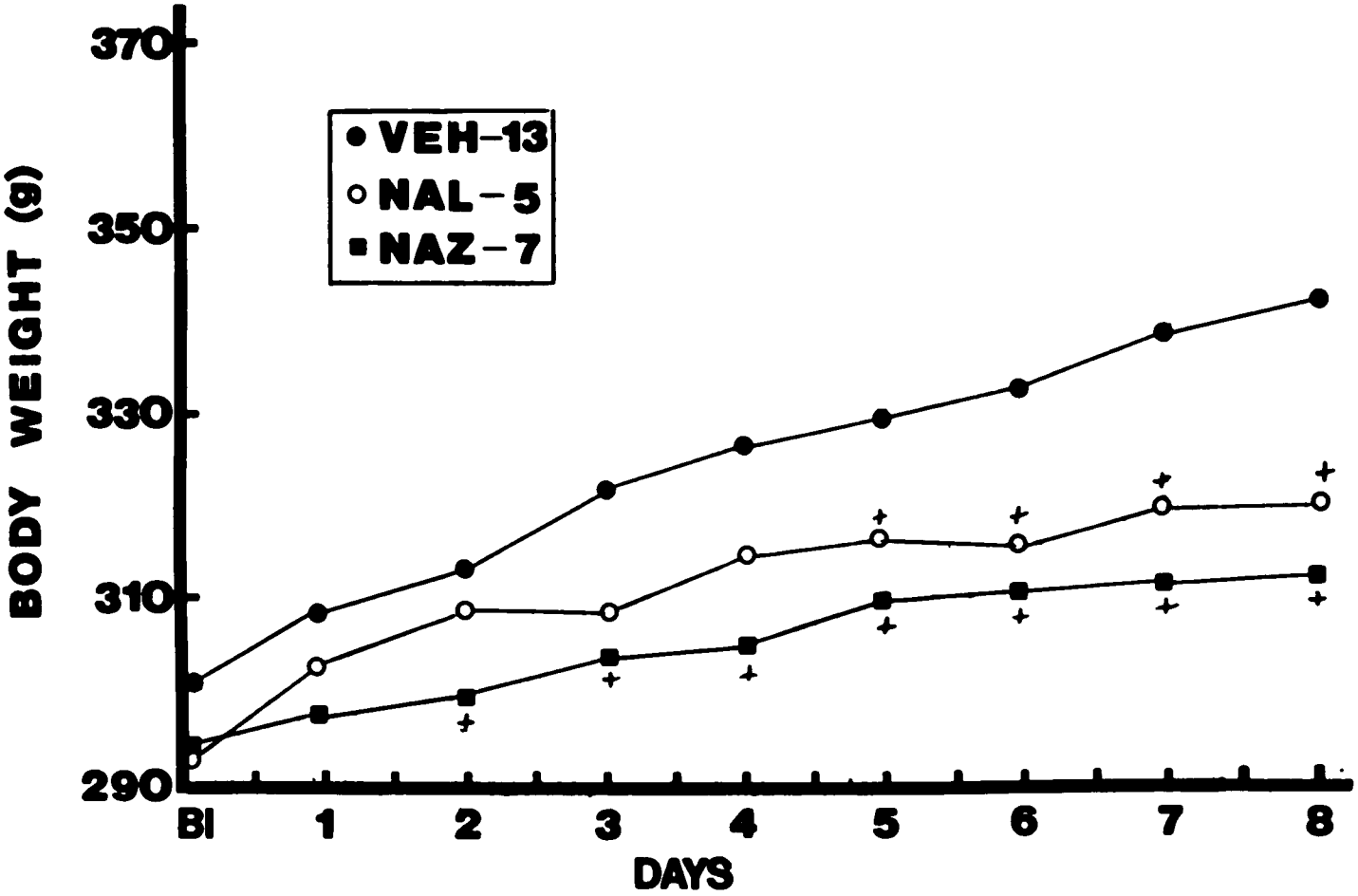

370
350
330
310
290
BODY WEIGHT (g)
• VEH-13
○ NAL-5
▪ NAZ-7
BI
1
2
3
4
5
6
7
8
DAYS

Figure 7. Body weight difference scores (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 14-day injection regimen in adolescent rats (B1 - baseline; + significantly less than vehicle (Dunnett comparison, P<.05)

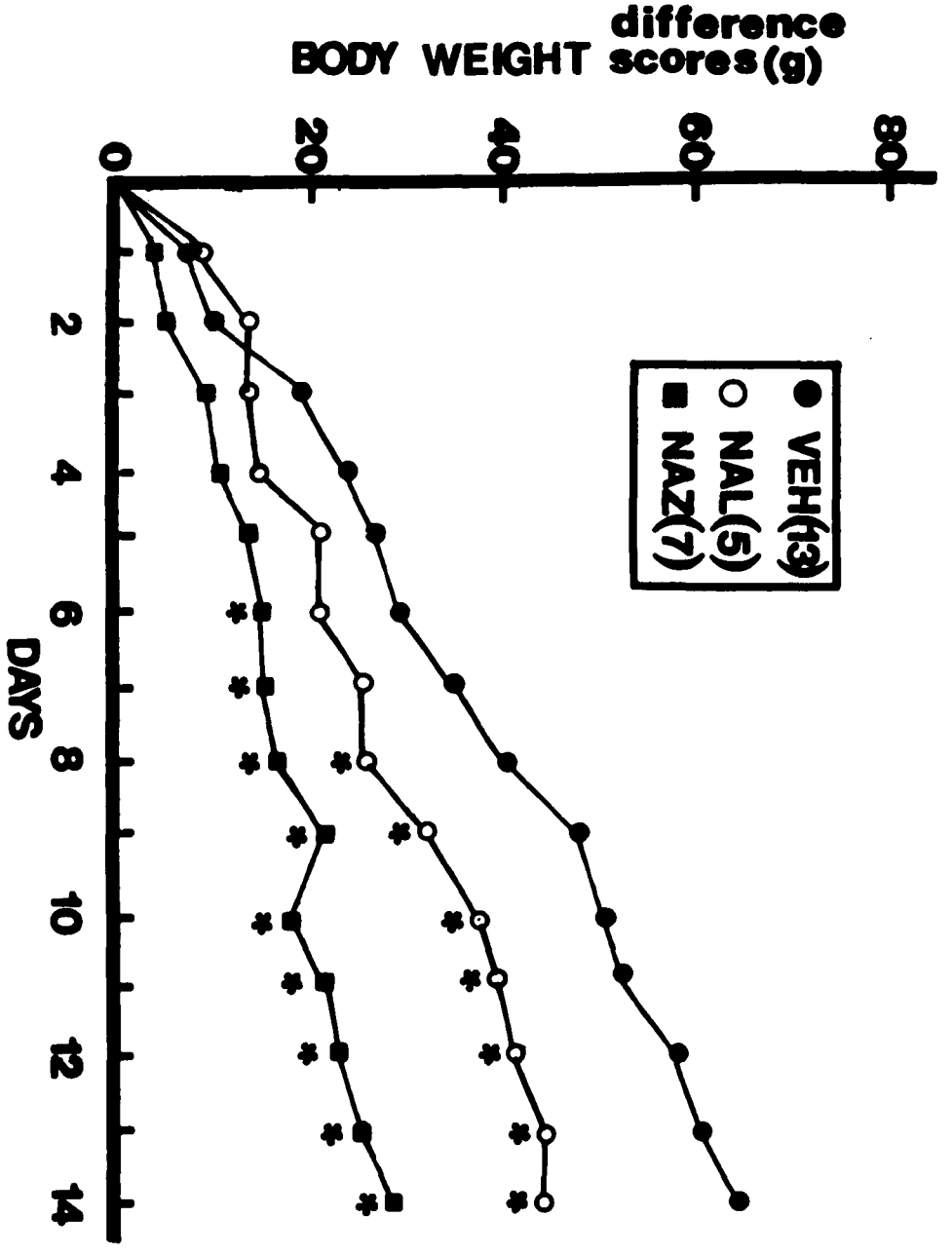
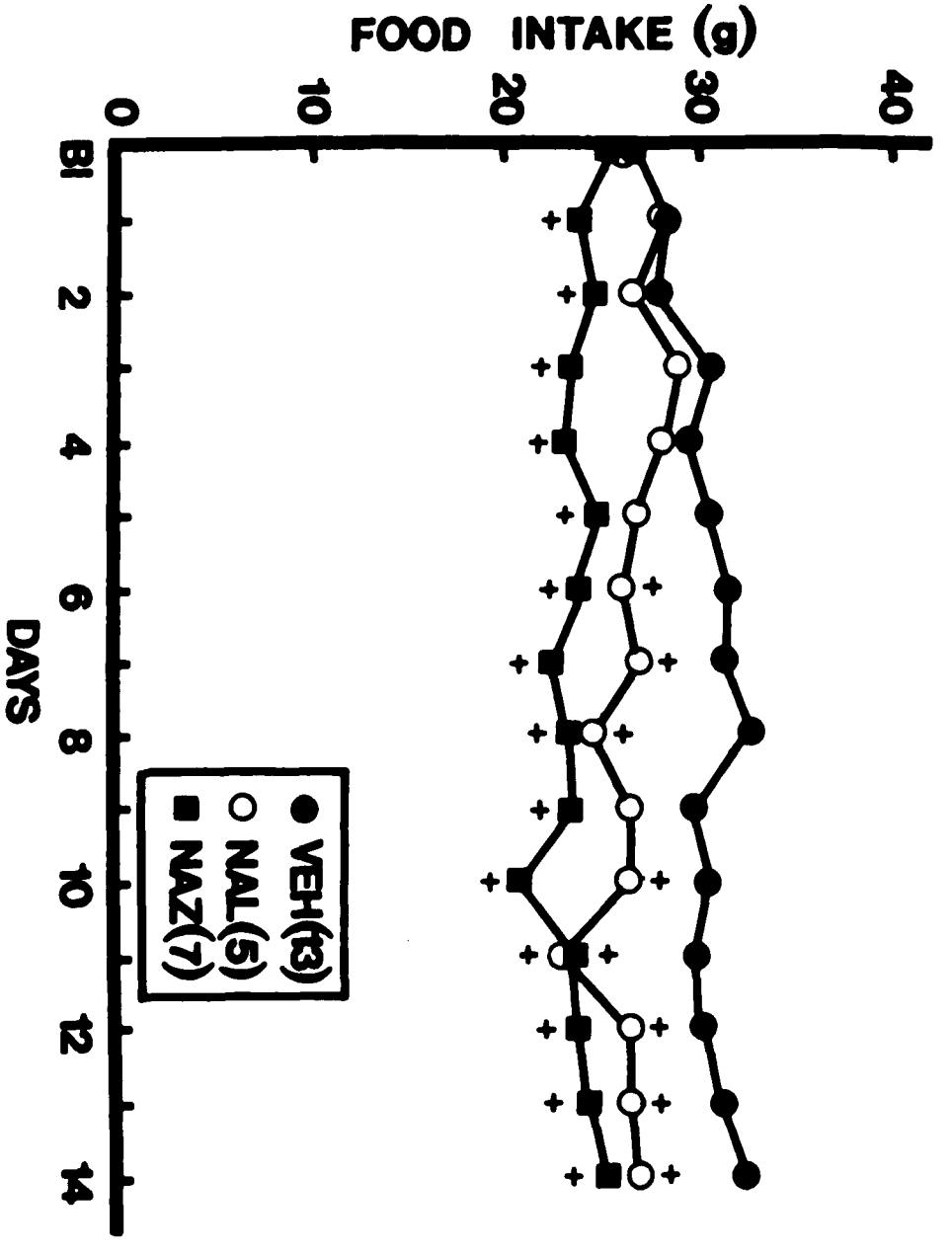

BODY WEIGHT difference scores(g)
0
20
40
60
80
DAYS
2
4
6
8
10
12
14
VEH(13)
NAL(5)
NAZ(7)

Figure 8. Alterations in food intake (g) following chronic treatment with either vehicle, naloxone or naloxonazine over a 14-day regimen in adolescent rats (Bl - baseline; + significantly less than vehicle (Dunnett comparison, P<.05)).

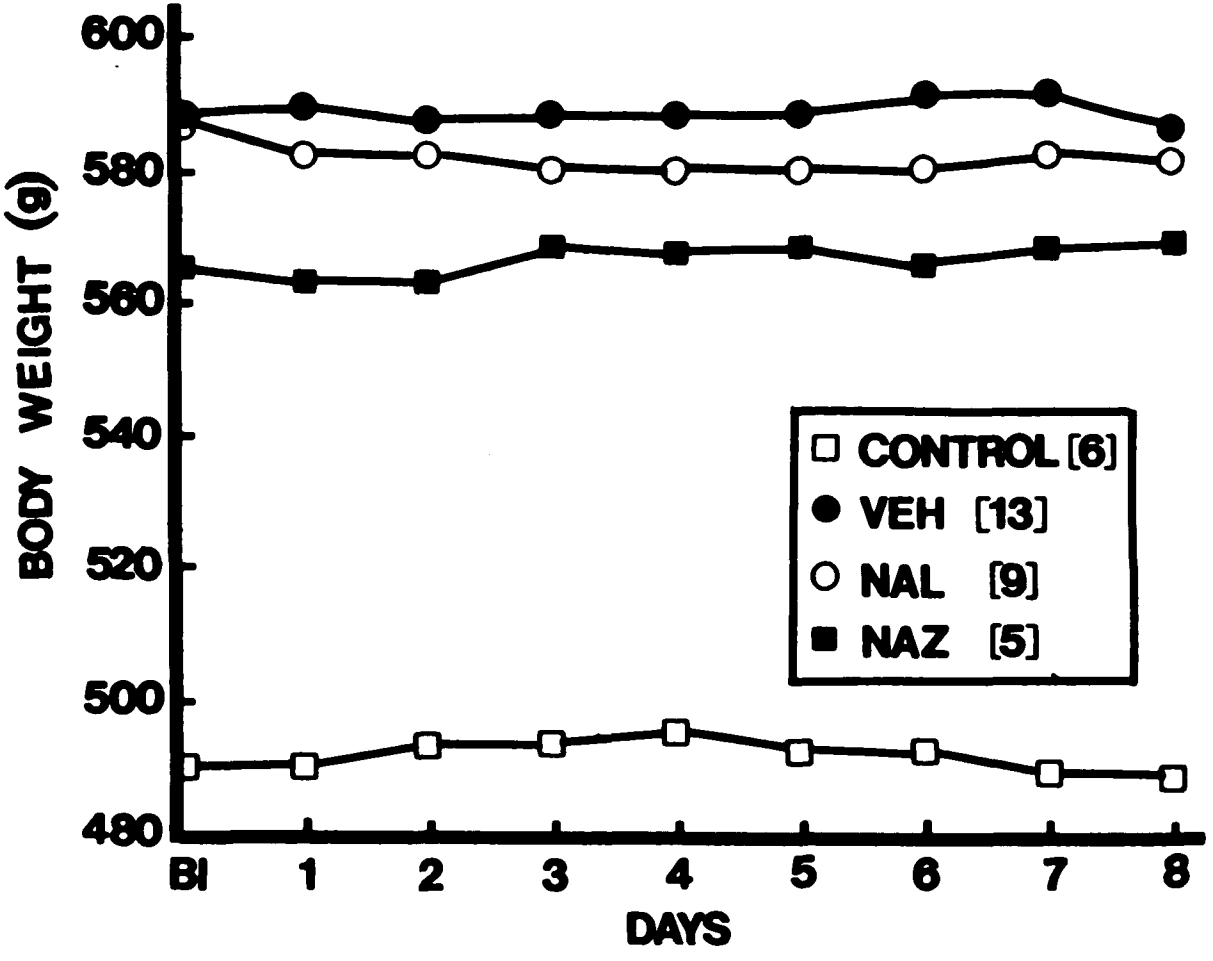

FOOD INTAKE (g)
0
10
20
30
40
BI
2
4
6
8
10
12
14
DAYS
● VEH(13)
○ NAL(5)
■ NAZ(7)

eighth and the tenth through fourteenth injections (Dunnett comparisons, P<.05). Naloxone treatment failed to alter intake relative to corresponding baseline values (99% of baseline), but reduced intake relative to corresponding vehicle values by 14%. Naloxonazine had an even greater effect on food intake. Naloxonazine-treated rats exhibited significant decreases in food intake relative to vehicle-treated rats across the entire 14-day regimen (Dunnett comparisons, P<.05). Naloxonazine treatment reduced intake relative to both baseline values (9% decrease) and corresponding vehicle values (20% decrease). Although the reduction in food intake was greater in naloxonazine-treated than naloxone-treated rats, the two groups failed to differ significantly from each other across the injection time course.

Correlations between daily changes in body weight and food intake indicated that these variables were not significantly related in either vehicle-treated adolescent rats (r(12)=-.224) or naloxone-treated adolescent rats (r(12)=.338). In contrast, retardation in body weight and food intake reductions were significantly related in naloxonazine-treated rats (r(12)=.636, P<.02) and accounted for 40% of the variance.

Dietary Obesity Protocol: Naloxone and naloxonazine failed to alter body weight in dietary-obese rats (Figure 9). Although signficant differences in body weight were observed among the control and all three dietary obese groups (F(3,29)=6.00, P<.003), body weight changes failed to occur across the time course (F(8,232)=0.15) or for

Figure 9. Failure of chronic treatment with either naloxone or naloxonazine to alter body weight (g) of dietary-obese rats over a 8-day regimen.


BODY WEIGHT (g)
600
580
560
540
520
500
480
BI
1
2
3
4
5
6
7
8
DAYS
CONTROL [6]
VEH [13]
NAL [9]
NAZ [5]

the interaction between groups and the time course ($F(24,232)=0.66$). The three dietary-obese groups were significantly heavier than the diet control group at the onset of testing (Dunnett comparisons, $P<.05$), and this relationship persisted across the 8-day regimen. Neither the naloxone-treated, naloxonazine-treated nor the two vehicle-treated groups displayed any changes in body weight relative to baseline values across the 8-day regimen (Dunnett comparisons, $P>.05$). This failure of naloxone and naloxonazine to affect body weight in dietary-obese rats was accompanied by a corresponding failure of either antagonist to affect mean consumption of the sweetened condensed milk.

Discussion

Confirming previous reports the opioid receptor agonists morphine, EKC, dynorphin and DADL each produced hyperphagia in rats under a variety of experimental conditions (Morley et al., 1983). The opiate agonist studies were undertaken to determine whether opiate-induced feeding was modulated through the mu-1 binding site. Each type of hyperphagia was blocked by the general short-acting opiate antagonist, naloxone but not by the specific irreversible mu-1 antagonist naloxonazine. Previous studies have shown that morphine, EKC, and DADL each produce supraspinal analgesia which can be blocked by naloxazone or naloxonazine thereby implicating the mu-1 binding site in opiate mediated analgesia (Pasternak, 1980; Pasternak et al., 1980b; Zhang & Pasternak, 1981b). Previous studies have also implied a role for the mu-1 binding site in feeding behavior. Both naloxone and naloxonazine inhibit free-feeding and deprivation-induced feeding in rats (Simone et al., 1985). The results of the opiate agonist studies presented here were unexpected in light of these previous reports. Naloxonazine failed to affect the hyperphagic actions of morphine, EKC, dynorphin and DADL suggesting that opiate-induced feeding is not mediated through the mu-1 receptor.

The chronic opiate antagonist studies were undertaken to determine the involvement of the mu-1 binding site in body weight maintenance and chronic food intake in three different feeding models: the adult rat, the adolescent rat in a stage of dynamic

growth and the rat made obese by exposure to a 'cafeteria' diet. Chronic treatment with either the non-selective opiate antagonist naloxone or the mu-1 selective antagonist naloxonazine produced significant reductions in body weight and food intake in adult rats and significant retardations in body weight gain and food intake in adolescent rats, demonstrating mu-1 mediation of these effects. In light of previous reports showing that naloxone either failed to produce long-term effects on body weight or food intake (Frenk & Rosen, 1979; Pfeiffer, Nikolarakis, & Pfeiffer, 1984) or transiently decreased intake and then was subject to tolerance (Brands et al., 1979; Olson et al., 1985a; Ostrowski et al., 1981; Shimomura et al., 1982), naloxone's long-term effects on body weight and food intake in both feeding models in the present study were unexpected. Also unexpectedly, chronic treatment with either naloxone or naloxonazine failed to alter body weight and food intake in rats made obese and subsequently maintained on a cafeteria diet.

The following three sections will discuss in detail the relationship of these findings to previous reports and the implications of the results in terms of physiological mechanisms and clinical relevance.

1. The Mu-1 Receptor and Opioid-induced Feeding.

The opiate agonist studies confirmed previous reports that food intake could be stimulated following administration of putative agonists of the mu (Jalowiec et al., 1981; Sanger & McCarthy, 1980), kappa (Morley & Levine, 1981b; Morley et al., 1982a,b; McLean &

Hoebel, 1983) and delta (Tepperman & Hirst, 1983) opioid receptor subtypes. When morphine was administered to mildly food-deprived rats, it stimulated feeding by 25-32% over a 4 h period as compared to vehicle injections. As expected from previous studies (e.g., Sanger & McCarthy, 1981), naloxone completely blocked the hyperphagic effect of morphine, reducing intake to within vehicle levels. If the mu-1 receptor was involved in the mediation of this supposed mu-selective effect, naloxonazine would also be expected to reduce or eliminate morphine hyperphagia. In fact, naloxonazine failed to reduce morphine hyperphagia but rather potentiated this effect under the dose and experimental conditions employed. Pasternak and colleagues have provided many instances in which a physiological, pharmacological or behavioral effect of morphine has been shown to be mediated by the high-affinity mu-1 binding site. The mu-1 selective antagonists, naloxazone and its -azine derivative, naloxonazine, block such morphine effects as supraspinal analgesia, catalepsy, prolactin release, hypothermia and acetylcholine turnover (Pasternak & Wood, 1986). In contrast, neither mu-1 antagonist alters such morphine effects as spinal analgesia, growth hormone release release, respiratory depression, sedation, bradycardia, lethality, dopamine turnover, endotoxic shock and some signs of physical dependence. Like the latter group of effects, it would appear that the stimulatory effects of morphine upon food intake is mediated by either the low-affinity mu-2 binding site or another opioid receptor subtype (kappa, delta or sigma).

Indeed, Morley and co-workers (Morley et al., 1984) have hypo-

thesized that central kappa opiate receptors may be mediating opioid-induced feeding. The results of the present studies using EKC and dynorphin, both putative kappa receptor agonists, appear to provide further support for this hypothesis. Both EKC (47-51% increases) and dynorphin (122% increase) administered to freely-feeding rats produced an even greater hyperphagic effect than morphine, effects which agree with previously-reported studies (Morley et al., 1982b; Morley & Levine, 1981b). The non-selective antagonist, naloxone completely blocked the hyperphagic effect of EKC at the 2 mg/kg dose but failed to alter it at the 5 mg/kg dose. If one assumes that the pharmacological effects of EKC and morphine are at the kappa and mu receptors, respectively, the ability of naloxone to differentially affect hyperphagia induced by these agonists at equimolar (5 mg/kg) doses is predictable since naloxone has been shown to be less potent at the kappa receptor than at the mu receptor (Morley et al., 1984). Naloxonazine, on the other hand, failed to affect EKC-induced hyperphagia at either of the 2 mg/kg and 5 mg/kg doses, again indicating that the mu-1 binding site appears not to be involved in this response. The results of the dynorphin experiment were very similar: intracerebroventricular administration to freely-feeding rats stimulated food intake which was completely blocked by naloxone, but not by naloxonazine. These results lend support to the hypothesis that dynorphin is the endogenous ligand mediating opioid involvement in normal feeding behavior and that the kappa receptor may be the site of action. Unfortunately, the only way that relative kappa involvement in the opioid modulation of food intake can be tested is through

comparison of the relative potencies of kappa and other opioid agonists (see reviews: Cooper et al., 1985; Morley et al., 1983, 1985). The reason for this is that there are few selective kappa antagonists. For instance, MR 2266, a proposed kappa antagonist, displays some degree of cross-reactivity with mu binding sites. It should be noted however that the mu-1 site and the kappa sites display little cross-reactivity in binding studies (Pasternak, personal communication). As with morphine, EKC actions can be divided into mediation by mu-1 sites and non-mu-1 sites. Pasternak (1980) previously demonstrated that the analgesic actions of systemic EKC, like morphine was blocked by the mu-1 antagonist, naloxazone; the present study found that systemic EKC hyperphagia, like morphine hyperphagia was unaffected by mu-1 antagonism. The hyperphagic effect of dynorphin is also non-mu-1 mediated. Although any mu-1 mediation of dynorphin analgesia has not been evaluated, it is interesting to note that this form of analgesia is most consistently elicited following direct spinal administration (Goldstein et al., 1979) and not supraspinally. In contrast, the mu-1 site mediates supraspinal, but not spinal forms of analgesia (Ling & Pasternak, 1983). Rather, kappa and delta sites appear to mediate spinal opioid analgesia (see reviews: Yaksh, 1981; Basbaum & Fields, 1984).

The delta receptor and its putative delta endogenous ligands, the enkephalins may also be involved in food intake. DADL, a long-lasting leu-enkephalin analogue, also produced robust hyperphagia (236% increase) when administered intracerebroventricularly confirming previous reports (Tepperman & Hirst, 1983). Systemic

naloxone completely blocked the hyperphagic effect of DADL. In contrast, naloxone injected into the ventromedial hypothalamus failed to affect DADL-induced hyperphagia (Tepperman & Hirst, 1983) suggesting that this site is not responsible for the effect. Once again, naloxonazine failed to affect DADL-induced increases in food intake indicating that the mu-1 binding site is not involved in this response. A delta-receptor mechanism is rather implied since ICI 174,864, a potent delta receptor antagonist is capable of eliminating DADL hyperphagia (Jackson & Sewell, 1985).

Opiate and opioid peptide stimulation of feeding is similar to and different from the stimulation of feeding by other neurotransmitters. Norepinephrine-induced feeding is predominantly an increase in carbohydrate ingestion rather than protein or fat (Leibowitz, Weiss, Yee, & Tretter, 1985). Opiates, on the other hand, potentiate protein and fat intake (Marks-Kaufman, 1982). Both norepinephrine and opiates inhibit firing of the paraventricular nucleus of the hypothalamus (PVN), the putative site of action of both these substances in inhibiting the "satiety" actions of the PVN (Moss, Urban, & Cross, 1972; Pittman, Hatton, & Bloom, 1980). Opiates and opioid peptides also stimulate feeding when injected into the perifornical area in addition to the PVN unlike norepineprhine which stimulates feeding only in the PVN (Woods & Leibowitz, 1985). Neuropeptide Y produces a very potent stimulation of feeding. When it is injected into the PVN a sated rat will eat in 4 h what it will normally it in 24 (Stanley & Leibowitz, 1985). The opiate-induced feeding described in the above experiments was robust but was not as intense as the

stimulation of feeding by neuropeptide Y.

The reduction by naloxone, but not naloxonazine of opioid-induced feeding appears to be the result of selective and not non-specific effects for the following reasons. First, naloxone eliminated the hyperphagic effects of morphine, EKC, dynorphin and DADL by reducing intake within levels observed in vehicle-treated rats. It did not abolish feeding completely which might be expected from effects of non-specific malaise. Second, the same doses of naloxone and naloxonazine are active in other feeding models: both naloxone and naloxonazine at 10 mg/kg reduce free-feeding and deprivation-induced feeding. Therefore, any reduction by naloxone, but not naloxonazine upon opioid-induced feeding was not due to the former's reduction of feeding per se given the effectiveness of both antagonists to decrease free feeding. Third, others have shown that naloxone-induced anorexia is not due to either general motor disruption (Carey et al., 1981) or drug-induced aversion (Leshem, 1984; Ostrowski, Foley, Ling, & Reid, 1980; Wu, Cruz-Morales, Quinan, Stapleton, & Reid, 1979). Finally, any difference observed between the effects of naloxone and naloxonazine upon morphine hyperphagia could not be attributed to the route of administration or the time interval between injection and testing. Both subcutaneous and intravenous naloxone administered 5 min prior to morphine produced similar inhibition of the hyperphagic response, an interval consistent with its pharmacological duration of action. In contrast, naloxone failed to affect morphine-induced hyperphagia when administered 24 h prior to the agonist, an interval in which its reversible pharmacological

actions are dissipated. Naloxone and a naloxonazine analogue also have different effects in another unrelated system. As mentioned above, naloxone but not naloxazone, a similar irreversible, long-lasting mu-1 antagonist to naloxonazine, blocks endotoxic shock (Holaday et al., 1983). Naloxazone failed to prevent the hemodynamic effects of endotoxic shock which can be blocked by naloxone and other opiate receptor antagonists.

To summarize, comparison of the antagonistic actions of naloxone and naloxonazine provides insight into the involvement of the mu-1 binding site in opioid-mediated behaviors. While reversal of an effect by naloxone defines opioid action, reversal of an effect by both naloxone and naloxonazine suggests involvement of the mu-1 binding site. Since naloxone but not naloxonazine blocked the hyperphagic effects of morphine, EKC, dynorphin and DADL, it appears that opioid-induced feeding is not mu-1 mediated. This was suprising since the mu-1 binding site appears to mediate the supraspinal analgesic responses induced by these same agonists (Pasternak, 1980; Pasternak et al., 1980a,b; Zhang & Pasternak, 1981b). Thus, the receptor or binding site subpopulations responsible for the opioid mediation of ingestive behavior on the one hand, and supraspinal analgesia on the other appear to differ. This extends our knowledge of which opiate actions are mediated by the mu-1 binding site (e.g., supraspinal analgesia, catalepsy, prolactin release, hypothermia, acetylcholine turnover, free-feeding and deprivation-induced feeding) and which are non-mu-1 mediated (e.g., spinal analgesia, growth hormone release, respiratory depression, sedation, bradycardia,

morphine lethality, dopamine turnover, endotoxic shock, some signs of physical dependence, glucoprivic feeding and opioid-induced feeding).

Based on previous reports and the results of the present experiments it appears that the mu-1 binding site is involved in free-feeding and deprivation-induced feeding. Glucoprivic feeding and feeding induced by the opioid agonists, morphine, EKC, dynorphin and DADL appears to be non-mu-1-mediated. It is possible that glucoprivic and opioid-induced feeding may be mediated by the kappa opioid receptor but this research awaits the development of very specific kappa antagonists. The delta opioid receptor may also be involved in feeding. The delta receptor antagonist, ICI 174,864 has interesting similarities to naloxonazine in that it reduces free-feeding, yet has no effect on glucoprivic feeding (Simone et al., 1985; Jackson & Sewell, 1985). However, naloxonazine fails to affect DADL hyperphagia, while ICI 174,864 reduces this hyperphagic response (Jackson & Sewell, 1985). The effect ICI 174,864 has on feeding induced by other opiate receptor subtypes is unknown. Further experiments should examine different opiate receptor antagonists, such as ICI 174,864, beta-funaltrexamine (beta-FNA) which selectively alkylates the mu receptor (Ward, LoPresti, & James, 1986; Takemori, Ikeda, & Portoghese, 1986), and beta-chlornaltrexamine (beta-CNA), a derivative of naltrexone which alkylates all opiate receptors (Portoghese, Larson, Jiang, Takemori and Caruso, 1979; James & Goldstein, 1984) to determine their effects on different feeding models (e.g., free-feeding, deprivation-induced feeding.

The failure of naloxonazine to affect glucoprivic feeding

(Simone et al., 1985) and opioid-induced feeding (see above) may indicate that the mu-1 binding site is involved in 'long-term' ingestive conditions but not in either 'short-term' regulatory challenges (e.g., glucoprivation) or stimulatory conditions (e.g., opioid-mediated hyperphagia).

2. The Mu-1 Receptor and Chronic Effects on Body Weight and Food Intake in Adult and Adolescent Rats.

Chronic treatment with either naloxone or naloxonazine produced significant reductions in body weight and food intake in adult male rats across a 14-day regimen. In general, naloxone and naloxonazine did not differ from each other in their ability to reduce body weight and food intake in the adult rat. Naloxonazine produced a 7% decrease in body weight and a 22% decrease in food intake, while naloxone decreased body weight by 4% and reduced food intake by 13%. In adolescent rats, chronic treatment with either naloxone or naloxonazine significantly retarded body weight gain and reduced food intake in a similar 14-day injection paradigm. Naloxonazine retarded body weight gain by 53% and reduced food intake by 27%. Naloxone had a significantly smaller effect, retarding weight gain by 33% and reducing food intake by 14% over the injection regimen.

Naloxonazine's effects on body weight and food intake might be predicted a) if the mu-1 binding site is involved and b) since naloxonazine is a long-lasting, irreversible opiate receptor antagonist that has been shown to reduce free-feeding 24 h subsequent to injection (Simone et al., 1985). That naloxone was capable of

producing significant and persistent decreases in body weight and food intake over a chronic injection paradigm was somewhat unexpected in light of several previous reports. Although some studies (Brands et al., 1979; Jalowiec et al., 1981; Margules et al., 1978,1979) have shown that naloxone treatment does reduce body weight and food intake in a chronic injection regimen, others have either failed to observe an effect (Frenk & Rosen, 1979; Pfeiffer et al., 1984) or have found that any decreases were transitory and subject to a form of tolerance; body weight initially decreased but then returned to normal levels after several days (Brands et al., 1979; Olson et al., 1985a; Ostrowski et al., 1981; Shimomura et al., 1982). The present chronic administration study differs from the above-mentioned negative studies in several respects. First, the effects of naloxone on body weight and food intake were cumulative, initially appearing after several injections and then producing a gradual and progressive alteration over the 14-day regimen. Second, naloxone and naloxonazine were administered intravenously which increases the possiblity that maximal amounts of the drug reaches relevant central target structures. In previous studies, naloxone was administered either intraperitoneally or subcutaneously which allows part of the drug to be sequestered and/or eliminated from relevant areas.

The reduction of body weight and food intake in adult and adolescent rats appears to be specific to maintenance of weight and intake rather than due to nonspecific malaise or changes in water exchange. The body weight loss in adult rats and the retarded body weight gain in adolescent rats were gradual rather than abrupt.

Also, food intake showed stable decreases following each antagonist rather than aphagia typically associated with sickness or malaise. Earlier studies have shown that moderate doses of opiate receptor antagonists do not produce conditioned taste aversions (Leshem, 1984; Ostrowski et al., 1980; Wu et al., 1979). Further evidence that naloxonazine does not produce malaise can come from the results of the opiate agonist studies. Naloxonazine failed to affect EKC, dynorphin, and DADL-induced hyperphagia and even potentiated morphine-induced hyperphagia. If naloxonazine produces aversion it would then be expected to block food intake in these conditions as well as acute and chronic free-feeding conditions. In addition, naloxone and naloxonazine had no effect on food intake and body weight in dietary obese rats (see next section) which are usually very sensitive to aversive stimuli. Opiate receptor antagonists may affect renal water and electrolyte excretions which could then account for the observed reductions in food intake. However, Lang and co-workers have shown that while chronic administration of naltrexone reduced food and water intake, it had no effect on electrolyte balance or water exchange (Lang, Strahlendorf, Strahlendorf, Lutherer, & Barnes, 1982).

Chronic opiate antagonist treatment also affects opioid binding sites and morphine-induced analgesia (Yoburn, Goodman, Cohen, Pasternak, & Inturrisi, 1985; Yoburn, Cohen, & Inturrisi, 1986). Rats implanted with naltrexone pellets for 8 days show a 45% increase in whole brain radiolabelled opioid-binding 24 h after pellet removal which is accompanied by a 50% increase in the analgesic effect of

morphine. This effect is not seen after a 24 h implantation. The "up-regulation" that is observed after naltrexone pellet removal may be relevant to the anorectic effect of chronic naloxone and naloxonazine treatment. If chronic antagonist treatment produces a "down-regulation" during the actual treatment this may mean that the endogenous opioid ligands that may be involved with food intake have fewer opioid-binding sites to bind to and therefore food intake is inhibited.

The effects of naloxone and naloxonazine were more pronounced in adolescent rats than in adult rats. This may be due to the effects of the opiate receptor antagonists on the endocrine system. Both exogenous (e.g. morphine) and endogenous (e.g. beta-endorphin) opioids have been shown to produce effects on hypothalamo-pituitary function (Morley, 1981; Howlett & Rees, 1986). Systemic or central administration of beta-endorphin, met-enkephalin and morphine in rats stimulate growth hormone release (Dupont, Barden, Cusan, Merand, Labrie, & Vaudry, 1980; Martin, Audet, & Saunders, 1975). This effect is reversed by naloxone. Naloxone alone has not been shown to have a definitive effect on growth hormone release. Meites and coworkers (Bruni, VanVugt, Marshall, & Meites, 1977) have shown that naloxone reduces growth hormone release but this has not been replicated (Martin et al., 1975). Opiates and opioid peptides also affect the release of gonadotrophins, prolactin, thyroid-stimulating hormone, ACTH, vasopressin and oxytocin (Howlett & Rees, 1986). The effects of opiate receptor antagonists alone on hypothalamo-pituitary function have not been as well documented, but their actions on such

systems may explain why both naloxone and naloxonazine exerted a greater effect on body weight and food intake in adolescent rats as compared to adults.

Determining the site of action of the opiate receptor antagonists can suggest possible mechanisms of action. As mentioned before, opiate receptors are distributed throughout the central nervous system and body (e.g., Atweh & Kuhar, 1977a,b,c), including areas usually associated with feeding behavior such as the hypothalamus, neostriatum, amygdala, and gastrointestinal tract. The kappa opiate receptor subtype has been found particularly in areas known to be involved in taste and feeding such as the rostral pole of the nucleus of the solitary tract, parabrachial nuclei, medial hypothalamus, medial nuclei of the amygdala and bed nucleus of the stria terminalis (Lynch, Watt, Krall, & Paden, 1985). Recent evidence has shown that various opiate agonists and antagonists increase or decrease feeding behavior only when injected into specific brain regions (Gosnell, Morley, & Levine, 1986; Woods & Leibowitz, 1985). Dynorphin increased food intake only when injected into the paraventricular (PVN) and ventromedial (VMH) nuclei of the hypothalamus but not the lateral hypothalamus or the globus pallidus. Naloxone decreased food intake only when injected into the PVN, VMH and globus pallidus but not the lateral hypothalamus or the striatum (Gosnell et al., 1986). When morphine was injected into the PVN and the perifornical hypothalamus (PFH), it produced a strong feeding response which did not occur when injected into the VMH. Naloxone, on the other hand, while effective in the PVN and PFH also produced a reduction in feeding

when injected into the VMH. This contrasts with the effects of norepinephrine (NE). NE produced a feeding response when injected into the PVN but not the PFH (Woods & Leibowitz, 1985).

The parallel reductions in body weight and food intake following chronic naloxone and naloxonazine treatment indicate that the opioid modulation of these effects are mediated by the mu-1 binding site. The opioid modulation of body weight maintenance and long-term control of food intake by the mu-1 binding site is similar to mu-1 mediation of free feeding and deprivation-induced feeding observed following acute naloxone and naloxonazine treatment (Simone et al., 1985). It differs from the ability of naloxone but not naloxonazine to inhibit hyperphagia induced by glucoprivation (Simone et al., 1985) and as described above hyperphagia induced by morphine, EKC, dynorphin and DADL.

Chronic administration of naloxone and naloxonazine was effective in reducing body weight and food intake under "long-term" conditions implicating the mu-1 binding site in ongoing body weight maintenance and feeding behavior. In contrast, naloxonazine was ineffective under "short-term" regulatory challenges such as glucoprivation and opioid-mediated hyperphagia suggesting that these conditions are non-mu-1-mediated.

3. The Mu-1 Receptor and Dietary Obesity.

Chronic treatment with naloxone and naloxonazine failed to affect body weight or milk intake in rats made obese on a cafeteria diet. This is in marked contrast to the reduced weight and retarded

weight gain observed in adult and adolescent rats, respectively. The failure of the opiate antagonists to have an effect in obese rats was quite surprising. Previous evidence has shown opioid involvement in the preference for palatable diets in normal rats and intake in obese rats. Margules and co-workers (1978) initially found that genetically obese rats (fa/fa) and genetically-obese mice (ob/ob) were more sensitive to naloxone than lean littermate controls, and that pituitary beta-endorphin concentrations were elevated in obese rats and mice, suggesting that these increased concentrations were involved in the development of obesity. This hypothesis was supported by observations that rats made obese by ventro-medial hypothalamic lesions (King et al., 1979) and genetically-obese Zucker rats (McLaughlin & Baile, 1983, 1984) were more sensitive to naloxone than lean controls. However, this hypothesis has been challenged by the failure to observe increased sensitivity to naloxone or increased beta-endorphin concentrations in some non-genetic models of obesity. Gunion and Peters (1982) examined the effects of naloxone on the development of obesity in rats with ventromedial hypothalamic lesions, dorsolateral tegmental lesions and parasagittal hypothalamic knife cuts, manipulations which usually produce obesity. Naloxone suppressed intake in all groups equally. In this study, however, the animals were food-restricted to prevent the occurrence of the obesity and all experimental and control animals were within a normal weight range. The ability of acute pretreatment of naloxone to decrease preferences for palatable or sweet diets is well-established (e.g., Cooper, Barber, & Barbour-McMullen, 1985; Cooper, Jackson, Morgan, &

Carter, 1985; Lowy et al., 1980; Levine et al., 1982), as is the ability of acute pretreatment of naloxone to decrease intake of a cafeteria diet (Apfelbaum & Mandenoff, 1981; Mandenoff, Fumeron, Apfelbaum, & Margules, 1982). Moreover, chronic administration of naloxone zinc tannate, a salt which releases naloxone slowly over several days prevented the occurrance of dietary obesity (Mandenoff, et al., 1982). A major difference between the present study and that of Mandenoff's is that our animals were made dietarily-obese before the onset of chronic naloxone or naloxonazine treatment. Thus, a number of different procedural factors appear important in the opioid modulation of dietary obesity. Short-term effects of opiate antagonists appear to reliably block preferences for sweet, palatable or high-caloric foods. Chronic antagonist treatment affects the development of dietary obesity, it does not reduce weight or reduce intake in animals that are already obese. A similar effect was observed in Zucker rats that are genetically obese (McLaughlin & Baile, 1983). Nalfemene, a highly-potent opiate antagonist, reduced body weight gain. Dietary obesity in rats has been used as an animal model of some forms of environmentally-induced human obesity (Sclafani & Springer, 1974). Opiate receptor antagonists such as naloxone and naltrexone have been used in both clinical settings and with normal subjects. Naloxone reduced intake in patients with the Prader-Willi syndrome, a condition which produces obesity at an early age (Kyriakides, Silverstone, Jeffcoate, & Laurance, 1980). In normal human volunteers of normal body weight, a single intravenous injection of naloxone, reduced food intake as compared to placebo

(Trenchard & Silverstone, 1983). There was no change on subjective ratings of hunger, satiety, mood, arousal or on total fluid intake. In contrast, naltrexone at a dose of 200 mg/day failed to reduce body weight in a ten week trial of males and females who were 30-100 percent overweight (Malcolm, O'Neil, Sexauer, Riddle, Currey, & Counts, 1985). Maggio and co-workers (Maggio, Presta, Filippo Bracco, Vasselli, Kissileff, Pfohl, & Hashim, 1985) investigated the effect of naltrexone on spontaneous eating behavior of eight moderately obese male volunteers for 28 days in a hospital setting. The subjects received placebo, 100, 200, or 300 mg/day doses of naltrexone in a repeated measures, cross-over design. Naltrexone failed to reduce food intake at any dose although the smallest dose (100mg) produced a slight reduction in body weight. Therefore the clinical utility of naloxone and other opiate antagonists as anorectics is still in doubt. Naloxone reduces gastric secretion both baseline levels and meal-induced while having no effect on the secretion of pancreatic polypeptides (Feldman, Walsh, & Taylor, 1980). Experiments should be performed examining the effect of chronic administration of long-lasting, irreversible antagonists such as naloxonazine on the physiology of the intestinal hormones, ACTH, cortisol and related hormones.

The results described above on the effects of opiate antagonists in humans are consistent with the effects described in rats. Naltrexone reduced intake in normal subjects but had no effect in obese situations. Naloxone and naloxonazine reduced intake and body weight in adult and adolescent normal weight rats but had no effect on rats

that had already been made obese. Since the effectiveness of opiate antagonists as potential anorectics is questioned under the present paradigm of dietary-induced obesity, it is important to determine the precise factors under which different opiate antagonists produce anorectic effects. Future experiments could examine the degree of development of dietary obesity which renders opiate antagonist-induced anorexia ineffective and/or the role of dietary constituents offered to the animal during antagonist treatment. It is possible that a certain level of obesity alters the effective dose for opiate antagonist anorexia or the continuous accessibility of highly-palatable and caloric foods may offset the potentially transient anorectic effects of opiate antagonists.

In summary, chronic treatment with naloxone and naloxonazine failed to alter body weight and milk intake in dietary obese animals that are maintained on their palatable diet. This indicates that body weight maintenance and possibly food intake in a dietary obese situation is mediated by nonmu-1 binding sites as is glucovprivic feeding and feeding induced by the opioid peptides. This contrasts with the loss of body weight and reduction in food intake in adult rats and the reduction in body weight gain and food intake in adolescent rats which is thought to be mu-1 mediated similar to the mu-1 mediation of free-feeding and deprivation-induced feeding.

General Discussion

It has been known for centuries that opiates produce a variety of behavioral and physiological effects such as analgesia and euphoria. Morphine has also been used medicinally for its effect on the gastrointestinal system in the control of diarrhea. The existence of endogenous opioids in areas of the brain that are involved in ingestion (e.g., hypothalamus) and peripherally in the vagus nerve and that opioids reduce gastric motility thereby slowing food transit has reinforced speculation that the endogenous opioids may be involved with the normal regulation of food intake. The significance of the endogenous opioids in normal feeding behavior and appetite regulation is still controversial. It is possible that opioids are involved in metabolic changes which lead to food intake rather than in hunger and appetite per se. Morley (1981) has suggested that the endogenous opioids and their hormonal effects are involved with an organism's response to stress or the phenomenon of "flight or fight". Beta-endorphin is secreted from the pituitary concomittantly with ACTH in stress-related situations. The effect endogenous opioids have on feeding behavior may be a secondary event to maintain energy balance. It has also been proposed that endogenous opioids are involved with the adaptation to pending famine, to maintain homeostasis (Margules, 1979). Shortage of food would lead to activation of the endogenous opioid system in order to initiate feeding, to lay down fat and conserve nutrients and ions. Energy would be conserved

by limiting general activity as well as reduced reproductive activity. At the extreme this would lead to hibernation. Many studies have implied that endogenous opioids may be involved with the sensory qualities of ingestion (Reid, 1985). The differential effect naloxone has on food that supposedly tastes better may be due to its effect on the "pleasantness" of the food. Endogenous opioids may affect the hedonic features of certain stimuli. Naloxone's anti-dipsogenic effect is potentiated by sweet or salty flavors while morphine increases intake of preferred saccharin solutions.

The discovery of the involvement of endogenous opioids in ingestion is a relatively recent phenomenon. In addition to endogenous opioids, monoamines and neuropeptides may be involved in the neurochemical and neuroendocrinological regulation of appetite. Alpha-adrenergic stimulation in the PVN induces feeding while beta-adrenergic stimulation of the LH inhibits feeding (Leibowitz, 1980). Certain neuropeptides have been found to either stimulate or inhibit feeding. While CCK, bombesin, calcitonin and neurotensin reduce food intake, pancreatic polypeptide, neuropeptide Y, peptide YY and endogenous opioids increase food intake. However, there are differences in the effects these neuropeptides have on food intake. CCK and bombesin appear to act both peripherally as well as centrally to decrease feeding. Endogenous opioids stimulate feeding but only under very specific conditions involving dose, time intervals and state of deprivation. Opioids also produce a short-lived stimulation of intake. This contrasts with the effects of neuropeptide Y which can stimulate intake such that an animal's normal daily intake will

occur in a 2-4 h period. Thus, opioid control of feeding is definitely not exclusive but one of many endogenous systems regulating food intake. It is important for future studies to evaluate the interaction of the opioid system with other neuronal systems involved in ingestion. To do this however, we must identify specific receptor subtypes involved in different forms of opioid feeding. The preceeding series of experiments have expanded our knowledge of this area. Finally, given the interesting constellations of behaviors and physiological effects that are mediated by mu-1 and non-mu-1 actions, we can begin to understand how opiates in general alter underlying homeostatic states.

Appendix

Table of Abbreviations

ethylketocyclazocine	EKC
D-ala^2-D-leu^5-enkephalin	DADL
naloxone	NAL
naloxonazine	NAZ
BL	baseline
i.v.	intravenous
s.c.	subcutaneous
i.c.v.	intracerebroventricular

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