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NALOXONAZINE, A HIGH-AFFINITY OPIATE RECEPTOR ANTAGONIST: EFFECTS UPON ANALGESIC AND INGESTIVE PROCESSES

by

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ABSTRACT

Naloxonazine, a High-Affinity Opiate Receptor Antagonist: Effects Upon Analgesic and Ingestive Processes

by

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One primary method in investigating the involvement of endogenous opioids in such behaviors as analgesia and ingestion is to observe behavioral changes following administration of opiate antagonists. The primary opiate antagonist employed in behavioral pharmacological research has been naloxone. However, naloxone does not compete equally well for all opiate receptor sub-types, and therefore may not be an ideal antagonist for assessing opioid involvement in behavior. Naloxonazine is a long-lasting opiate antagonist which selectively inhibits MU1 binding sites. Morphine analgesia is eliminated by naloxonazine (10 mg/kg) intravenously (iv) administered 24 h prior to the opiate. To examine central effects of this antagonist, the first experiment evaluated the dose-response relationships of intracerebroventricular (icv) administration of naloxonazine upon basal pain thresholds and morphine analgesia. Groups of six male albino Sprague-Dawley rats, matched for baseline tail-flick latencies and jump thresholds, failed to display changes in these measures for up to 24 h following the central (1, 5 or 50 ug, icv) injections. However, central naloxonazine pretreatment displayed dose-dependent

and test-dependent effects in reducing or eliminating morphine analgesia 24 h later.

The second experiment examined the effects of intravenous and central naloxonazine administration upon freely feeding, food deprived and glucoprivic rats. Rats matched for baseline food intake received an iv injection of either naloxonazine (10 mg/kg) or vehicle. Food intake was assessed 24 h later. Pretreatment with naloxonazine significantly reduced free feeding relative to vehicle treatment. In contrast, central naloxonazine pretreatment failed to reduce free feeding over a 24 h period. In a second study, food intake of six groups of rats deprived of food for 24 h, was assessed 2 and 24 h thereafter. Pretreatment with iv naloxonazine, but not naloxone, prior to deprivation, significantly reduced deprivation-induced intake at 2 h following food reinstatement. Naloxone was effective at reducing intake only when administered shortly (5-15 min) before food reintroduction. In contrast, central pretreatment with naloxonazine (50 ug) prior to deprivation, significantly reduced intake at 24 h, but not at 2 h, after food reinstatement. In a third study, three groups of rats received 2-deoxy-D-glucose (2-DG) (400 mg/kg, ip) either 24 h following naloxonazine (10 mg/kg, iv) or vehicle, or immediately following naloxone (10 mg/kg, sc). Food intake was determined at 2 and 4 h following 2-DG administration. Naloxone significantly reduced 2-DG hyperphagia at 2 and 4 h, and naloxonazine potentiated 2-DG hyperphagia at 4 h following 2-DG administration.

Naloxonazine appears to be a powerful tool in assessing opioid involvement in behavior since it selectively blocks MU1 binding sites for well over 24 h.

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INTRODUCTION

Morphine and other opiate compounds have been used clinically for many years. Only recently, however, have findings suggested some of their basic biological mechanisms of action. New approaches emerged in the study of opiates and their physiological and behavioral consequences following the discovery of the opiate receptor in 1973, including receptor blockade with opiate antagonists. Naloxonazine is a recently developed opiate antagonist which selectively blocks a specific sub-class of opiate receptors (MU1) at very low doses. The purpose of the present studies was to evaluate the behavioral effectiveness of central and peripheral naloxonazine, and thereby the role of MU1 sites, in morphine analgesia and ingestive behaviors. A review of the literature on a) opiate receptors, b) opioid peptides, c) opiate receptor sub-types, d) opiate antagonists, and e) the role of opioids and their antagonists in behavior is provided.

A. OPIATE RECEPTORS

Numerous investigators had attempted to demonstrate the existence of opiate receptors in the brain, but such work was inconclusive until Goldstein, Lowney and Pal (1971) used a competitive binding assay to demonstrate the radiolabelled binding of the opiate agonist levorphanol to mouse brain homogenate. This effect, however, was non-stereospecific. Three separate groups of investigators (Pert and Snyder, 1973; Terenius, 1973; Simon, Hiller and Edelman, 1973) modified this radioactive labelling technique and demonstrated

stereospecific binding of the active levorotatory (L) isomer of opiates to brain membranes. Opiate receptor binding has since been demonstrated in a number of species, including fish, birds, reptiles, amphibians and mammals (Pert, Aposhian and Snyder, 1974). The distribution of opiate receptors in humans, monkeys and rats is similar (Kuhar, Pert and Snyder, 1973; Pert and Yaksh, 1974; Pert et al., 1974) with the greatest density found in the amygdala. Other areas with high concentrations of opiate receptors include the striatum, thalamus, hypothalamus, midbrain periaqueductal gray (PAG), substantia nigra (SN), red nucleus, lateral reticular nucleus, solitary nucleus, area postrema and the substantia gelatinosa of the dorsal horn. In the peripheral nervous system, high concentrations of opiate receptors are found in the guinea pig ileum (Pert and Snyder, 1973) and the mouse vas deferens (Henderson, Hughes and Kosterlitz, 1973).

B. OPIOID PEPTIDES

Once the existence of opiate receptors was demonstrated, a search began for an endogenous compound that would bind to the opiate receptor as well as display the behavioral effects induced by opiate agents. Pasternak, Goodman and Snyder (1975) showed that brain extracts could compete with opiates for opiate receptor binding sites, while Hughes (1975) identified brain extracts which produced inhibition of the electrically-induced contractions of the guinea pig ileum and mouse vas deferens. This effect was identical to that of morphine and was blocked by low doses of the opiate antagonist

naloxone. These morphine-like substances were isolated in porcine brain (Hughes, Smith, Kosterlitz, Fothergill, Morgan and Morris, 1975) and were found to be two pentapeptides: methionine (met)-enkephalin (Tyr-Gly-Gly-Phe-Met) and leucine (leu)-enkephalin (Tyr-Gly-Gly-Phe-Leu). A second class of opioid peptides, the endorphins, were then isolated in the carboxyl terminus of the pituitary peptide beta-lipotropin hormone (B-LPH) (Li and Chung, 1976), alpha-endorphin (B-LPH: amino acids 61-76), beta-endorphin (B-LPH: amino acids 61-91), and gamma-endorphin (B-LPH: amino acids 61-77) (Chretien, Benjannet, Dragon, Seidah and Lis, 1976; Graf, Szekely, Ronai, Dunai-Kovacs and Bajsz, 1976; Lazarus, Ling and Guillemin, 1976). Met-enkephalin is present at the N-terminus of all of the endorphins (B-LPH 61-650 (Bradbury, Smyth, Snell, Deakin and Wendlandt, 1977), however its distribution is independent of these peptides (Watson, Akil, Richard and Barchas, 1978). A third class of endogenous opioid peptides, the dynorphins, were extracted from porcine pituitary (Goldstein, Tachibana, Lowney, Hunkapillar and Hood, 1979) and consist of 17 amino acids of which the first five are identical to leu-enkephalin (Goldstein, Fischli, Lowney, Hunkapillar and Hood, 1981). However, dynorphin is much more potent in the guinea pig ileum bioassay, and this suggests that dynorphin is not the precursor for leu-enkephalin. Indeed, the three classes of endogenous opioids appear to arise from three different precursors: the enkephalins arise from pre-proenkephalin (Kimura, Lewis, Stern, Rossier, Stein and Undenfriend, 1980), the endorphins arise from pro-opiomelanocortin (Mains, Eipper and Ling, 1977), and dynorphin

arises from pre-prodynorphin (Kakidani, Furutani, Takahashi, Noda, Morimoto, Hirose, Asai, Inayama, Nakanishi and Numa, 1982).

Immunocytochemical analysis indicates that the distribution of the enkephalins and beta-endorphin show a general dissociation. The enkephalins have been found intrinsically in cells and fibers of the globus pallidus, caudate nucleus, putamen, hypothalamus, thalamus, SN, PAG, nucleus raphe magnus (NRM), locus coeruleus (LC), amygdala, hippocampus and the substantia gelatinosa of the spinal cord (Finley, Lindstrom and Petrusz, 1981; Hokfelt, Elde, Johansson, Terenius and Stein, 1977; Pickel, Sumal, Beckley, Miller and Reis, 1980; Simantov, Kuhar, Uhl and Snyder, 1977; Uhl, Goodman, Kuhar, Childers and Snyder, 1979). The enkephalins are also present in the intermediate lobe of the pituitary gland, the adrenal medulla, cerebrospinal fluid, plasma and throughout the digestive system.

In contrast to the widespread distribution of the enkephalins, beta-endorphin is contained within a well-defined pathway origin originating in the arcuate nucleus of the hypothalamus (Bloom, Battenberg, Rossier, Ling, Leppaluoto, Vargo and Guillemin, 1978). Fibers arising from these cells extend throughout the anterior hypothalamus and project to several midline structures, including the median eminence, periventricular nucleus of the thalamus, dorsal raphe nucleus, the PAG and the LC. Beta-endorphin and adrenocorticotrophic hormone (ACTH) are present in the same arcuate cells in the hypothalamus (Watson et al., 1978), thus keeping with their co-existence in the pro-opiomelanocortin precursor. Beta-endorphin is also present in the anterior and intermediate lobes of the pituitary

gland (Bloom, Bsttenberg, Rossier, Lidd and Guillemin, 1978), where it is stored and co-released with ACTH (Guillemin, Vargo, Rossier, Ling, Rivier, Vale and Bloom, 1977; Pelletier, Leclerc, Labrie, Coate, Chretien and Lis, 1977). In addition, beta-endorphin is present in plasma, the cerebrospinal fluid and small intestine (Schultz, Wuster and Hertz, 1977; Akil, Richardson, Barchas and Li, 1978; Forman, Sonntag, van Vugt and Meites, 1981; Jeffcoate, McLaghin, Hope, Rees, Ratter, Lowery and Besson, 1978).

Dynorphin, like the enkephalins, is widely distributed throughout the central nervous system although its distribution systems are not identical with those of the enkephalins (Goldstein and Ghazarossian, 1980; Watson et al., 1981). The highest levels of dynorphin are in the hypothalamus, particularly the paraventricular (PVN) and supraoptic nuclei where dynorphin coexists with vasopressin (Watson, Akil, Fischli, Goldstein, Zimmerman, Nilaver and van Weinersman, 1982). Dynorphin-containing perikarya are also found in other areas of the hypothalamus as well as the cerebral cortex, amygdala, PAG, nucleus solitarius, brain stem reticular formation and the dorsal horn of the spinal cord. In addition, dynorphin-containing fibers and terminals are found in the hypothalamus, caudate, putamen, globus pallidus, nucleus accumbens, hippocampus, SN pars reticulata, LC, NRM, reticular formation and the dorsal horn of the spinal cord (Khachaturian, Watson, Lewis, Coy, Goldstein and Akil, 1982). Dynorphin is also localized in the anterior and intermediate lobes of the pituitary gland as well as adrenal chromaffin cells (Goldstein and Ghazarossian, 1980).

C. OPIATE RECEPTOR SUB-TYPES

Opiate receptors are not homogeneous but, like the endogenous opioid ligands, can be categorized into several sub-classes (see review: Wood, 1982). This concept was initially based on studies of the actions of opiates in spinally transected dogs (Martin, Eades, Thompson, Huppler and Gilbert, 1977). They found that different opiate produce different behavioral and physiological responses, and that most of these effects are reversed by the opiate antagonist naloxone. Furthermore, while some opiates are capable of suppressing withdrawal symptoms in the morphine-dependent dog, others precipitate withdrawal. Maratin postulated the existence of at least three distinct opiate receptor sub-types named respectively for their prototype agonists: mu (morphine), kappa (ketocyclazocine) and sigma (SKF-10047: N-allylnorphenazocine). The guinea pig ileum and mouse vas deferens bioassays also provided evidence for the existence of multiple opiate receptors (Lord, Waterfield, Hughes and Kosterlitz, 1977) with potency differences among opiates in inhibition of electrically-induced contractions of these tissues. Kappa and mu receptor agonists are most potent in the ileum, while enkephalin is most potent in the vas deferens, suggesting the existence of a fourth type of opiate receptor, the delta receptor, for which enkephalin is the prototype agonist.

Biochemical and anatomical studies also support the notion of multiple opiate receptor sites. Competition and displacement studies using radiolabelled ligands and radiolabelled opiate antagonists have distinguished among mu, kappa and delta receptor sub-types (Chang and

Cuatrecasas, 1979; Chang, Cooper, Hazum and Cuatrecasas, 1979; Kosterlitz and Paterson, 1980). Displacement refers to the releasing of a bound ligand from the binding site. While morphine easily displaces radiolabelled morphine, it does not easily displace radiolabelled ketocyclazocine. Anatomically, the mu and delta receptors have been shown to be dissociated by examining the localization of opiate binding (Chang et al., 1979; Goodman, Snyder, Kuhar and Young, 1980). Receptor binding studies have additionally revealed the presence of high and low-affinity binding sites for tritiated opiate agonists and antagonists (Pasternak and Snyder, 1975) and tritiated enkephalin (Lord et al., 1977). The term high-affinity (the degree of adhesiveness of the ligand to the receptor) is used when very low concentrations of a ligand display a large amount of receptor binding. The term low-affinity refers to the additional binding which takes place only after much higher concentration of the ligand is added to the assay solution.

D. OPIATE ANTAGONISTS

Naloxone has been the most widely used antagonist in assessing opiate activity in behavioral, pharmacological and biochemical studies (see review: Sawynok, Pinsky and LaBella, 1979). However, multiple opiate receptor sub-types exist and naloxone may therefore not be the ideal pharmacological tool for either purpose. Naloxone does not compete equally well for all opiate receptor sub-types, displaying a high affinity for the mu receptor, but competing poorly for the other opiate receptor sub-types (Lord et al., 1977). Therefore, the use of

naloxone makes it difficult to assess the role in behavioral of the other opiate receptor sub-types and the endogenous opioid ligands that may selectively and preferentially occupy these receptors. Furthermore, naloxone has a short duration of action and must be given 5-30 min prior to opiate administration.

Recently, new opiate antagonists have been developed which block specific opiate receptor sub-types and have a long duration of action (Pasternak, Childers and Snyder, 1980a; Hahn, Buatti and Pasternak, 1982). Naloxazone, a hydrazone derivative of naloxone, blocks for durations over 48 h the high-affinity component of binding sites for the mu receptor ligands naloxone and dihydromorphine (Pasternak et al., 1980a), the delta receptor ligand enkephalin (Wolozin and Pasternak, 1981), the kappa receptor ligand ethylketocyclazocine (Pasternak, 1980) and the sigma receptor ligand SKF-10047 (Pasternak, Carroll-Buatti and Spiegel, 1981). Naloxazone and naloxone can compete similarly for the high-affinity component of the mu receptor; however, naloxazone's longer duration of action makes it a more powerful tool. Naloxazone administered 24 h prior to either opiates or opioid peptides attenuates their analgesic effects in a dose-dependent manner without affecting opiate-induced lethality (Pasternak et al., 1980a, Pasternak, 1980; Wolozin and Pasternak, 1981). This suggests that high-affinity binding to receptors is necessary for the full expression of opiate and opioid peptide analgesia. Naloxazone's inhibition of high-affinity binding of all prototype opiate receptor agonists and the resultant elimination of opiate analgesia has led Pasternak, Childers and Snyder, (1980b) to

suggest that the high-affinity binding sites represent one class (MU1) of opiate receptors which mediate opiate analgesia. Another more potent antagonist, naloxonazine, was subsequently developed and displays similar pharmacological and biochemical actions to naloxazone but at much lower doses (Hahn et al., 1982). Like naloxazone, naloxonazine injected intravenously eliminates morphine analgesia 24 h after its administration (Ling, Spiegel, Nishimura and Pasternak, 1983), the time when inhibition of MU1 binding is maximal.

E. OPIOIDS AND BEHAVIOR

Two approaches were employed to study the roles of opiate receptors and opioid peptides in behavior: a) observe the behavioral effects following peripheral or central administration of opiate agonists or the peptides themselves, and b) observe the behavioral effects following opiate receptor blockade induced by opiate antagonists. It is possible to assess the tonic involvement of opioid peptides in behavior as well as the effects following opiate administration by blocking the receptors. These types of studies have revealed that the opioid systems are involved in a variety of behavioral and physiological responses, including sexual behavior (McIntosh, Vallano and Barfield, 1980; Myers and Baum, 1979), reward mechanisms (Belluzzi and Stein, 1977), learning and memory (Kastin, Scollan, Ehrensing, Schally and Coy, 1978; Stein and Belluzzi, 1979), thermoregulation (Yehuda and Kastin, 1980), cardiovascular responses (Florez and Mediavilla, 1977; Holaday, 1982) and respiration (Florez and Mediavilla, 1977). The involvement of opioid systems in pain

perception and ingestive behavior, which is the primary focus of the present experiments, is detailed below.

Opioid systems and pain perception: Reynolds (1969) found that electrical stimulation of the PAG produced a powerful analgesia (stimulation produced analgesia: SPA) in rats. This effect was extended to other subcortical structures including the NRM (Oleson and Liebeskind, 1975; Oliveras, Redjemi, Guilbaud and Besson, 1975), lateral hypothalamus (Balagura and Ralph, 1972; Rose, 1974) and LC (Sandberg and Siegel, 1977). Naloxone was initially reported to block SPA by some laboratories (Akil, Mayer and Liebeskind, 1976; Oliveras, Hosobuchi, Redjemi, Gilbaud and Besson, 1977), but not others (Pert and Walter, 1976). However, recent work has indicated that the site stimulated plays a vital role in determining naloxone antagonism in that SPA elicited from ventral, but not dorsal PAG sites displays naloxone-reversible effects (Cannon, Prieto, Lee and Liebeskind, 1982; Prieto, Cannon and Liebeskind, 1983). In addition, microinjections of morphine into the PAG produces analgesia which is reversed by naloxone (Jacquet and Lajtha, 1976; Lewis and Gebhart, 1977; Sharpe, Garnett and Cicero, 1974). Intracerebroventricular administration of the enkephalins (Belluzzi, Grant, Garsky, Sarantakis, Wise and Stein, 1976; Tseng, Ostwald, Loh and Li, 1977) and beta-endorphin (Bradbury et al., 1977; Ueda, Amano, Seiomi and Takagi, 1979), like morphine, produces naloxone-reversible increases in pain thresholds. Repeated administration of beta-endorphin produces tolerance to the analgesia and cross-tolerance to morphine (Tseng, Loh and Li, 1976). Microinjections of enkephalin and beta-endorphin into the subarachnoid

space of the spinal cord increase pain thresholds (Yaksh, Huang, Rudy and Frederickson, 1977; Yaksh and Henry, 1978) as does central administration of the opioid peptide dynorphin (Petrie, Tiffany, Baker and Dahl, 1982; Przewlocki, Sherman and Hertz, Spencer, 1976). Systemic administration of the enkephalins elicits a weak analgesia (Buscher, Hill, Roemer, Cardinaux, Closse, Hauer and Pless, 1976; Plotnikoff, Kastin, coy, Christensen, Schally and Sprites, 1976) while intravenous administration of beta-endorphin produces a potent analgesia, though at much higher doses than central injections (Tseng, Loh and Li, 1976).

Pain inhibition can be the consequence of either overactivation of a tonically-active system or phasic activation of a normally quiescent system. If the opioid system operates in a tonic manner, blockade of opiate receptors by opiate antagonists should interfere with such tonic function and thereby induce hyperalgesia. However, naloxone has been reported to induce either analgesia (Sewell and Spencer, 1976), hyperalgesia (Carmody, Carroll and Morgans, 1979), biphasic changes in pain thresholds (Woolf, 1980a; Woolf, 1980b) or no effects at all (Goldstein, Pryor, Otis and Larsen, 1976; North, 1977; Weisenfeld and Hallin, 1983). Systemic naloxone at doses of 5 mg/kg and higher increases tail-withdrawal latency in both mice and rats on the tail immersion test at water temperatures of 48-55^{o}C (Sewell and Spencer, 1976). Systemic naloxone doses of 50 or 100 ug/kg decrease hindpaw withdrawal latencies in mice and rats in response to electrical stimulation (Carmody et al., 1979). Pain thresholds as measured by either the tail-immersion (Woolf, 1980a), shock escape (Goldstein et

al., 1976) or formalin (North, 1977) tests have been shown to be unaffected by systemic naloxone administration. Furthermore, chronic systemic infusion of naloxone (80 or 800 ug/h) fails to alter hot plate latencies (Weisenfeld and Hallin, 1983).

Central administration of naloxone also produces inconsistent effects upon pain thresholds. Small though significant increases in tail-withdrawal latencies are observed on the tail-immersion test following naloxone injections into the lateral (10 ug or higher) and third (150 ug) ventricles, but not into the PAG or NRM (Sewell and Spencer, 1976; Woolf, 1980a). Biphasic alterations in tail-immersion thresholds occur following intrathecal administration of naloxone with a 7.5 ug dose eliciting analgesia, and 50 or 150 ug doses producing hyperalgesia (Woolf, 1980b). Furthermore, while the 7.5 ug naloxone dose fails to alter shock vocalization thresholds following intrathecal administration, a 50 ug dose produces hyperalgesia. Failure of central injections of long-lasting opiate antagonists to alter basal pain thresholds have also been reported. For example, intracerebroventricular administration of naloxazone at a dose of 50 ug failed to alter jump thresholds for up to 24 h after injection (Kirchgessner, Bodnar and Pasternak, 1982).

On the basis of stimulation and microinjection studies, Fields and Basbaum (1978) hypothesized an opioid-mediated pain inhibitory system which originates in the PAG, synapses in the NRM and projects to the dorsal horn of the spinal cord through the dorsolateral funiculus (DLF). Several lines of evidence support this hypothesis: a) SPA, as well as both systemic and central morphine analgesia, is blocked

following lesions placed in the DLF (Basbaum, Clanton and Fields, 1976; Murfin, Bennett and Mayer, 1976); b) lesions placed in the PAG or NRM attenuate morphine analgesia and SPA (Dostrovsky and Deakin, 1977; Garau, Mulas and Pepeu, 1975; Proudfit and Anderson, 1975; Samanin, Ghezzi, Mauron and Valzelli, 1973); c) sub-analgesic electrical stimulation summates with sub-analgesic doses of morphine to produce analgesia (Samanin and Valzelli, 1971); d) tolerance develops to SPA and SPA is partially cross-tolerant with morphine analgesia (Mayer and Hayes, 1975); and e) both electrical stimulation of and opiate microinjections into the PAG and NRM inhibit nociceptive neurons in the dorsal horn of the spinal cord (Oliveras, Besson, Guilbaud and Liebeskind, 1974; Ruda, Hayes, Price, Hu and Dubner, 1976).

An effort to define the conditions under which such a pain inhibitory system would be activated led to the finding that acute exposure to certain environmental stressors induces analgesia (stress-induced analgesia, or SIA) (Hayes, Bennett, Newlon and Mayer, 1976). Like SPA, not all forms of SIA are attenuated by naloxone or cross-tolerant with morphine (see review: Bodnar, 1983). For example, inescapable foot shock elicits analgesia that can be mediated by either opioid or non-opioid mechanisms as functions of a) the temporal parameters of the shock (Lewis, Cannon and Liebeskind, 1980), b) the number of shocks delivered (Grau, Hyson, Hyson, Maier, Madden and Barchas, 1981), and c) the body region shocked (Watkins and Mayer, 1982). Naloxone reverses the analgesia following prolonged intermittent shock (1-sec pulses delivered every 5 sec for 30 min),

but not that following brief continuous (3 min) shock (Lewis et al., 1980). Analgesia following 80 shocks, but not 20, shocks (5-sec pulses) delivered on a variable interval schedule is attenuated by naloxone (Grau et al., 1981). Furthermore, naloxone reverses analgesia following continuous shock (90-sec pulse) delivered to the forepaws, but has no effect on analgesia following shock to the hindpaws (Watkins and Mayer, 1982).

Opioid systems and ingestive behaviors: It is now apparent that the opioid peptides have a direct role in the modulation of ingestive behaviors (see review: Morley, Levine, Yim and Lowy, 1983). Alterations in feeding patterns have been observed following peripheral or central administration of opiate agonists, opioid peptides, and opiate antagonists. In addition, hyperphagic responses following various environmental manipulations are altered following the administration of opiate antagonists. Initially, it was reported that morphine-dependent rats increased food consumption following their morphine injections (Martin, Wikler, Eadesand and Pescor, 1963), and recent studies have revealed that systemic morphine also increases food intake in non-dependent rats (Jaloweic, Panksepp, Zolovick, Najam and Herman, 1981; Sanger and McCarthy, 1980). Opiate agonists, such as ketocyclazocine, ethylketocyclocine, cyclazocine and SKF-10047 also stimulate feeding following systemic administration (Morley, Levine, Grace and Kneip, 1982b; Morley et al., 1983; Sanger and McCarthy, 1981) suggesting that multiple opiate receptor sub-types may be involved. The hyperphagic effects of opiate agonists are reversed by naloxone (Sanger and McCarthy, 1981). Furthermore, feeding during the

night-time hours, the time of maximal feeding for rats, is decreased by naloxone (Brands, Thornhill, Hirst and Gowdey, 1979; Cooper, 1981; Jaloweic et al., 1981).

Central administration of opioid peptides have also been shown to induce consummatory behaviors. Intracerebroventricular administration of beta-endorphin (McKay, Kennedy, Edens, Williams and Woods, 1981) or dynorphin (Morley and Levine, 1981; Morley and Levine, 1982; Morley, Levine, Grace and Kneip, 1982b) increases food intake in satiated rats, an effect reversed by naloxone. Furthermore, injections of morphine (McLean and Hoebel, 1980; Tepperman, Hirst and Gowdy, 1981) or beta-endorphin (Leibowitz and Hor, 1980) into the ventromedial hypothalamus (VMH) or PVN of the hypothalamus stimulate feeding. These areas have previously been shown to be a primary anatomical site in the modulation of appetite and satiety (Leibowitz, 1980). Administration of norepinephrine or epinephrine into the hypothalamus stimulate ingestion (Grossman, 1962) through the alpha-noradrenergic receptors in the PVN (Slangen and Miller, 1969). There appears to be an interaction between beta-endorphin and norepinephrine in this feeding response since pretreatment with phentolamine, an alpha-adrenergic antagonist, blocks the feeding response following PVN injections of beta-endorphin (Leibowitz and Hor, 1980).

Studies employing genetically obese (ob/ob) mice and Zucker fatty (Fa/Fa) rats also demonstrate the involvement of endogenous opioids in the control of food intake in that elevated levels of beta-endorphin are found in the pituitary glands of both species and in the plasma of the obese rats (Margules, Moisset, Lewis, Shibuya and Pert, 1978). In

addition, these two species are more sensitive to naloxone's anorectic effects (Margules et al., 1978). It appears that an excess of beta-endorphin may be involved in the overeating since the hyperphagia is attenuated following hypophysectomy (Spiess, 1952).

Several environmental manipulations, including food deprivation, 2-deoxy-D-glucose (2-DG) administration and tail pinch stress also stimulate feeding in the rat. Holtzman (1974) first demonstrated the involvement of endogenous opioids in stress-related feeding by showing that naloxone decreases the hyperphagic response following food deprivation, an effect confirmed by others (Brown and Holtzman, 1979; Frenk and Rogers, 1979; Lowy, Maickel and Yim, 1980). 2-DG, by interfering with glucose metabolism, induces hyperphagia (Smith and Epstein, 1969) that is blocked by naloxone (Lowy et al., 1980; Sewell and Jawaharlal, 1980). Tail pinch in the rat reliably stimulates feeding (Antelman and Szechtman, 1975; Antelman, Szechtman, Chin and Fisher, 1975) which is decreased by naloxone (Morley and Levine, 1980).

RATIONALE

Opiate agonists, opiate antagonists and the endogenous opioids are implicated in pain perception and ingestive processes. In addition, there is anatomical, biochemical and pharmacological evidence which demonstrates the existence of several classes of opioid peptides and multiple opiate receptors. It is as yet unclear, however, whether and to what extent specific receptor sub-types and different opioid classes mediate nociceptive and ingestive behaviors. For example, while mu, kappa and delta opiate receptor agonists induce analgesia, mu, sigma and kappa receptor agonists alter nociceptive and feeding behaviors. In addition, opiate and opioid peptides display high and low-affinity binding which is most likely related to different receptor affinities. Because of the heterogeneity of opiate receptors and the fact that naloxone competes differentially for opiate receptor sub-types, antagonism by naloxone appears not to be the ideal method for ascertaining the role of endogenous opioids in behavior. Therefore, naloxonazine, a long-lasting and receptor-specific opiate antagonist, was employed in the present study to characterize further the basic opioid mechanisms involved in analgesia and ingestion.

The present studies evaluated: a) the dose-response and time course relationships of naloxonazine upon basal pain thresholds and morphine analgesia following its central administration; b) the involvement of high-affinity opioid binding sites in the control of food intake following naloxonazine administration; and c) the effects of central vs. peripheral naloxonazine administration upon feeding,

thereby suggesting central vs. peripheral mechanisms as a possible site of action. These studies characterized the basic opiate receptor mechanisms involved in opiate analgesia and in the control of food intake. Since one of the primary objectives of pain research is the development of potent analgesics without the undesirable side effects of presently used narcotics, understanding of the basic receptor mechanisms involved in pain inhibition is essential. In a similar fashion, characterizing the receptor mechanisms associated with ingestive processes is essential for the development of safe anorectics without side effects.

This dissertation will employ the terms "analgesia" and "hyperalgesia" which generally refer to respective increases and decreases in pain thresholds. These terms are defined operationally as increases or decreases in tail-flick latencies or jump thresholds.

GENERAL METHODS

Subjects: Adult male albino Sprague-Dawley rats (250-450 g) served as subjects. Animals were housed individually, maintained on a 12 h light 12 h dark cycle and were provided with food (Purina Rat Chow) and water ad libitum. In experiment 1, animals were matched across groups on the basis of their baseline tail-flick latencies and jump thresholds. In experiment 2, they were matched on the basis of their baseline food intake.

Intracerebroventricular (icv) cannulation: Rats were pretreated with chlorpromazine (3 mg/ml/kg body wt, IP) and anesthetized 20 min later with Ketamine hydrochloride (100 mg/ml/kg body wt, IM). A stainless steel 22 gauge guide cannula (Plastic Products) was stereotaxically implanted so that its 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, 1.5 mm lateral to the mid-sagittal suture and 3.6 mm from the top of the skull. Three stainless steel screws and dental acrylic secured the cannula to the skull. Animals were allowed a minimum of seven days to recovery from surgery.

Intravenous (iv) catheterization: Rats were anesthetized with Ketamine hydrochloride (100 mg/ml/kg body wt, IM) and supplemented with ether as necessary to maintain anesthesia. Since chlorpromazine can exert some physiological effects over a couple of days, and since

the testing protocol in the intravenously-catheterized rats occurred soon after surgery to insure the patency of the catheter, the chlorpromazine pretreatment was eliminated. An incision was made on the ventral surface of the neck, slightly to the right of midline. The fascia were separated and the jugular vein exposed and isolated. A 22 gauge needle pierced the vein and a vinyl intravenous catheter (Bo Labs; ID = 0.023 in, OD = 0.039 in) was inserted towards the heart (approximately 20 mm). The catheter was threaded under the animal's skin and through an opening on the dorsal surface of the neck for use in injections. Care was taken to insure that blood flowed easily through the catheter which was filled with heparin (50 U/ml) to prevent clotting. The catheter was closed by knotting it at the tip. Animals were allowed two days to recover from the surgery. Before and after all iv injections, heparin (0.3 ml) was flushed through the tubing.

Tail-flick test: Tail-flick latencies, a measure of reactivity to noxious heat (D'Amour and Smith, 1941), were measured by mounting a radiant heat source (IITC) 8 cm above the dorsum and 4 cm proximal to the tip of the tail of lightly restrained animals. When heat was applied to the tail, a timer was automatically activated. When the animal flicked its tail, a photocell beneath the tail was exposed which stopped the timer,and the latency to the nearest 0.01 sec was displayed. Previous work in our laboratory (Bodnar et al., 1984) has demonstrated that the temperature of the tail reaches the noxious range (49-53^{o}C: Price and Dubner, 1977) within 2.6-4.6 sec

following the onset of the thermal stimulus which explains latencies occurring below 3.5 sec. If an animal did not respond within 6 sec, the trial was automatically terminated to avoid tissue damage. The mean of four days of baseline tail-flick latencies were computed on the basis of daily means of three trials that were separated by 30 sec intertrial intervals.

Jump test: Jump thresholds, a measure of reactivity to electric shock (Evans, 1961), were determined by delivering electric shocks to the feet of unrestrained rats with a 60-Hz constant current shock generator (BRS/LVE) and grid scrambler (Campden Instruments) through a 30 x 24 cm floor composed of 16 grids. Using a modification of an ascending method of limits procedure, the jump threshold was defined in mA as the lowest of two consecutive intensities which elicited simultaneous removal of both hind paws from the grids. Each trial began with the animal receiving a 300 msec foot shock at a current intensity of 0.1 mA. Subsequent shocks were delivered at 5 sec intervals and increased in intensity in 0.05 mA steps until the jump threshold was determined. After each trial, the current intensity was reset to 0.1 mA and the procedure repeated until six trials were completed. The use of a static starting point in this study was to insure congruence with other experiments utilizing the jump test in our laboratory and those of others (see review: Bodnar, et al., 1980; Kelly, 1982). The static starting point in the jump test mimics the activation of heat delivered to the tail or feet in the tail-flick and hot-plate tests respectively. Work in this laboratory has

demonstrated repeatedly that the use of this static starting point does not result in learning as predicted by an error of anticipation. In contrast, the hot-plate test appears susceptible to learning effects (Simone and Bodnar, 1982). The ascending component of the method of limits procedure was employed to prevent the animal from receiving shock in the descending component that was well above the noxious range. Kelly (1982) has shown that exposure to supra-threshold values in one pain test can alter thresholds in a subsequent pain test. Four days of baseline jump thresholds were computed on the basis of the daily mean of these six trials. In all experiments, the order of tail-flick test followed by the jump test was chosen because this order minimizes carry-over effects of one pain test to another (Kelly, 1982).

Histology: Following experimental testing, an overdose of sodium pentobarbital was administered (200 mg/kg, ip) and animals underwent transcardiac perfusion with normal saline followed by 10% buffered Formalin. The brains were removed, blocked, sliced into 40 um coronal sections through the lateral ventricle, and stained with cresyl violet. Cannula placement was determined with a light microscope and only animals with a cannula properly placed in the lateral ventricle were included in the statistical analyses.

Statistical analyses: Analyses of variance (BMDP) were performed to determine whether any significant main or interaction effects occurred. Where applicable, Dunnett or Dunn comparison between

experimental and control groups and among time-matched pre- and post-injection conditions were performed to examine specific dose and time effects.

Drugs: Naloxonazine was synthesized in the laboratory of Dr. G.W. Pasternak, Memorial Sloan Kettering Hospital and Cornell Medical College, and was dissolved in normal saline and 0.2% acetic acid. Naloxone (Endo Laboratories) and morphine sulfate (Pennick) were dissolved in normal saline. Normal saline served as the vehicle solution for both agents. All icv injections were administered in a 10 ul volume through a 28 gauge internal cannula (Plastic Products) at a rate of 1 ul every 10 sec. All iv injections were administered in a 5 mg/ml concentration. In the morphine analgesia experiments, each animals received a maximum of one naloxonazine or naloxone injection and one morphine injection. In all experiments, naloxonazine was administered 24 h prior to any behavioral testing. This 24 h interval maximizes the selective biochemical antagonism of MU1 sites (Hahn et al., 1982). Short-term administration of naloxonazine results in non-selective opiate receptor antagonism similar to that of naloxone.

EXPERIMENT 1: Effects of Naloxonazine Upon Basal Tail-Flick Latencies, Jump Thresholds and Morphine Analgesia.

EXPERIMENT 1A: Central naloxonazine and basal pain thresholds.

Rationale: Since the discovery of opiate receptors and endogenous opioid peptides, a central question concerning the role of the endogenous opioid system in pain perception involves whether it is tonically active or phasically activated by stimulus situations that elicit analgesic responses. One means of testing tonic as compared to phasic influences is to determine whether administration of an opiate antagonist elicits a hyperalgesic effect in normal animals. To date, the use of naloxone in this paradigm has produced inconclusive results. While some studies have reported decreased pain thresholds following naloxone administration (Carmody et al., 1979, Woolf, 1980), others have reported no effects (Goldstein et al., 1976; North, 1977; Weisenfeld and Hallin, 1983). Moreover, in those cases when naloxone elicited hyperalgesia, the magnitude and duration of action were small, short and dependent upon the pain test employed. Furthermore, the longer-lasting opioid antagonist, naloxonazone, failed to alter baseline jump thresholds following central administration (Kirchgessner et al., 1982). In the present experiment, the effects of central administration of the opiate antagonist naloxonazine upon baseline tail-flick latencies and jump thresholds were examined over a 24 h post-injection time course.

METHOD

Twenty-four male cannulated rats were divided into four equal groups. Following central administration of either vehicle of 1, 5 or 50 ug doses of naloxonazine respectively, tail-flick latencies and jump thresholds were assessed at 0.5, 1, 2 and 24 h.

RESULTS

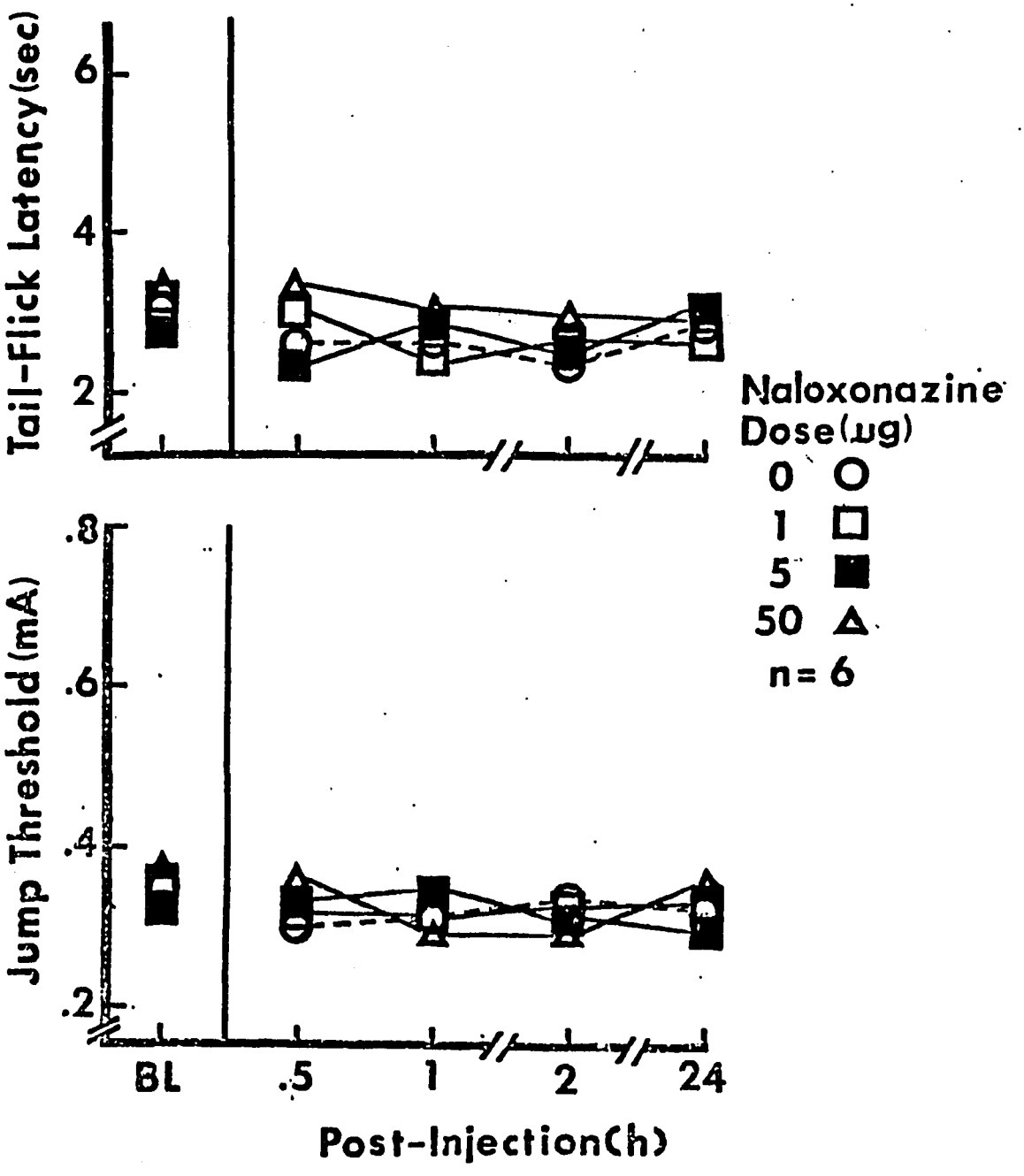
Insert Figure 1 here

Figure 1 shows that the 1, 5, or 50 ug doses of naloxonazine did not alter baseline tail-flick latencies or jump thresholds at either 0.5, 1, 2 or 24 h following central administration. While significant differences were observed across time for both tail-flick latencies ($F(4,80) = 5.64$, $p < .001$) and jump thresholds ($F(4,80) = 4.18$, $p < 0.004$), differences failed to occur among doses (tail-flick: $F(3,20) = 0.78$; jump: $F(3,20) = 0.00$) or for the interaction between doses and test times (tail-click: $F(12,80) = 1.20$; jump: $F(12,80) = 1.20$).

EXPERIMENT 1B. Central naloxonazine and morphine analgesia.

Rationale: To achieve maximal effectiveness in attenuating or eliminating morphine analgesia, naloxone must be administered either centrally or systemically 30 min or less prior to the opiate.

Figure 1. Failure of centrally administered naloxonazine (1, 5 or 50 ug) to alter tail-flick latencies (top panel) or jump thresholds (bottom panel). In this and all other figures, the measure of deviation of the mean (standard error) was found to be so small that it is encompassed by the size of the symbol used to indicate the mean.


Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
BL
.5
1
2
24
Post-Injection(h)
Naloxonazine Dose(µg)
0
1
5
50
n= 6

Although central (Kirchgessner et al., 1982) or systemic (Pasternak et al., 1980) administration of the long-lasting opiate antagonist naloxazone eliminates morphine analgesia when administered 24 h prior to the opiate, high doses are necessary. Lower doses of naloxonazine, the active component of naloxazone, have similar biochemical effects as naloxazone (Hahn et al., 1983) and antagonize morphine analgesia following iv administration (Ling et al., 1983). Since the previous study (Ling et al., 1983) employed only one naloxonazine dose and one morphine dose, a knowledge of full dose-response relationships between morphine and naloxonazine has not been established. Therefore, this experiment utilized a dose range of naloxonazine and a dose range of morphine to assess the ability of naloxonazine to attenuate morphine analgesia. Further, the present experiment employed an icv route of administration due to a limited availability of naloxonazine.

METHOD

One hundred and forty four cannulated rats were divided into twenty four equal groups. Animals received a central injection of either vehicle, or 1, 5 or 50 ug doses of naloxonazine 24 h prior to a subcutaneous injection of morphine sulfate (15, 10, 5, 2.5 or 0.5 mg/kg). Tail-flick latencies and jump thresholds were assessed immediately prior to and at 30, 60 and 120 min following morphine administration. Morphine (5 mg/kg) analgesia in the last four groups was evaluated over a 6 h post-injection time course, with tail-flick

latencies and jump thresholds assessed immediately prior to and at 0.5, 1, 2, 3, 4, 5 and 6 h after injection.

RESULTS

Figures 2-6 show the dose-response relationships of centrally-administered naloxonazine to morphine analgesia measured by the tail-flick and jump tests. Figure 2 shows that morphine analgesia, measured by the tail-flick test, induced by the highest (15 mg/kg) dose of morphine, was not antagonized by naloxonazine pretreatment. Significant differences in tail-flick latencies occurred across test times ($F(3,60) = 569.13$, $p < .001$) but failed to occur among doses ($F(3,20) = 0.78$) or for the interaction between doses and test times ($F(9,60) = 0.98$). Following the 10 mg/kg dose of morphine, tail-flick latencies were significantly altered across test times ($F(3,6 = 715.77$, $p < .001$) and approached significance among doses ($F(3,20 = 2.90$, $p < .06$) and for the interaction between doses and test times ($F(9,60) = 1.82$, $p < .08$). Dunnett comparisons revealed that only pretreatment with the 50 ug dose of naloxonazine significantly decreased morphine analgesia measured on the tail-flick test and only at 30 and 60 min following morphine (Figure 3). Figure 4 shows that with 5 mg/kg morphine, pretreatment with the 50 ug, but not the 1 and 5 ug naloxonazine doses, significantly decreased analgesia measured on the tail-flick test across the entire time course (Dunnett comparisons). Tail-flick latencies significantly differed among doses ($F(3,20) = 4.37$, $p < 0.16$), across test times (F

(3,60) = 276.55, $p < .001$) and for the interaction between doses and test times (F (9,60) = 4.44, $p < .001$).

Analgesia measured by the tail-flick test induced by a 2.5 mg/kg dose of morphine was eliminated in rats pretreated with 50ug naloxonazine dose (Figure 5). Tail-flick latencies significantly differed among doses (F (3,19) = 9.62, $p < .001$), across test times (F (3,57) = 49.16, $p < .001$) and for the interaction between doses and test times (F (9,57) = 3.27, $p < .003$). However, this analgesic effect was only attenuated at 120 min following morphine in animals pretreated with the 1 ug dose of naloxonazine (Dunnett comparisons). Indeed, a clear dose-response relationship for naloxonazine effects did not occur since pretreatment with 5 ug of naloxonazine failed to alter morphine analgesia. Figure 6 illustrates that the lowest (0.5 mg/kg) dose of morphine did not alter tail-flick latencies for any group. There were no significant differences in tail-flick latencies among doses (F (3,20) = 0.30), across test times (F (3,60) = 4.18) or for the interaction between doses and test times (F (9,60) = 0.88.

Insert Figures 2-6 here

While none of the naloxonazine doses altered analgesia measured on the tail-flick test induced by the 15 mg/kg dose of morphine, Figure 2 shows that the analgesia measured on the jump test induced by this dose was significantly attenuated at 30 and 60 min in rats pretreated with 1 and 50 ug of naloxonazine, and at 30 min in rats pretreated with 5 ug naloxonazine (Dunnett comparisons). Significant alterations

Figure 2. Reduction in morphine analgesia (15 mg/kg) 24 h following central naloxonazine pretreatment on the jump test but not on the tail-flick test. * indicates a significant difference from the vehicle-treated group, $p < .01$.

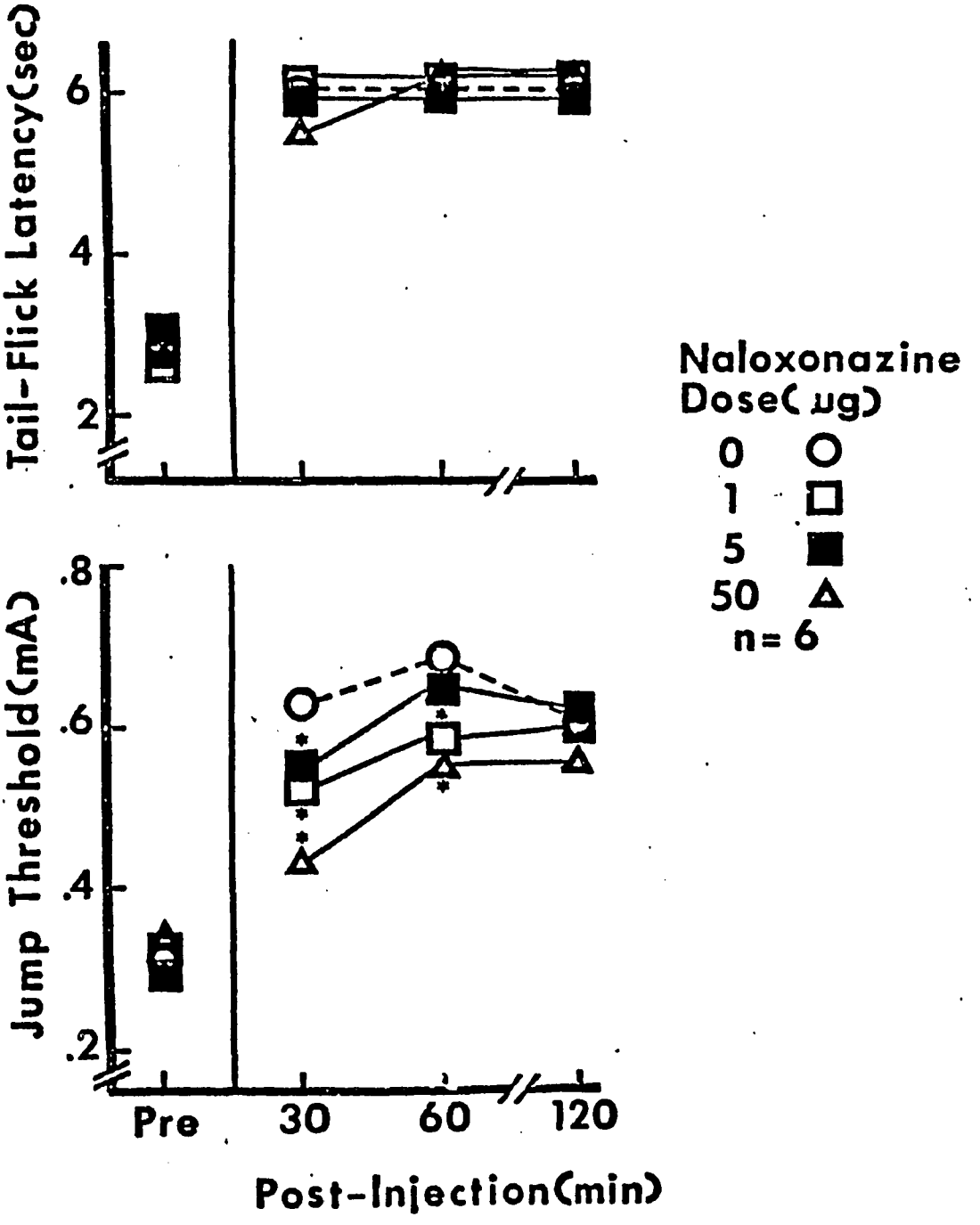

Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
Pre
30
60
120
Post-Injection(min)
Naloxonazine
Dose(µg)
0
1
5
50
n= 6

Figure 3. Reduction in morphine analgesia (10 mg/kg) 24 h following central naloxonazine pretreatment. * indicates a significant difference from the vehicle-treated group, $p < .01$).

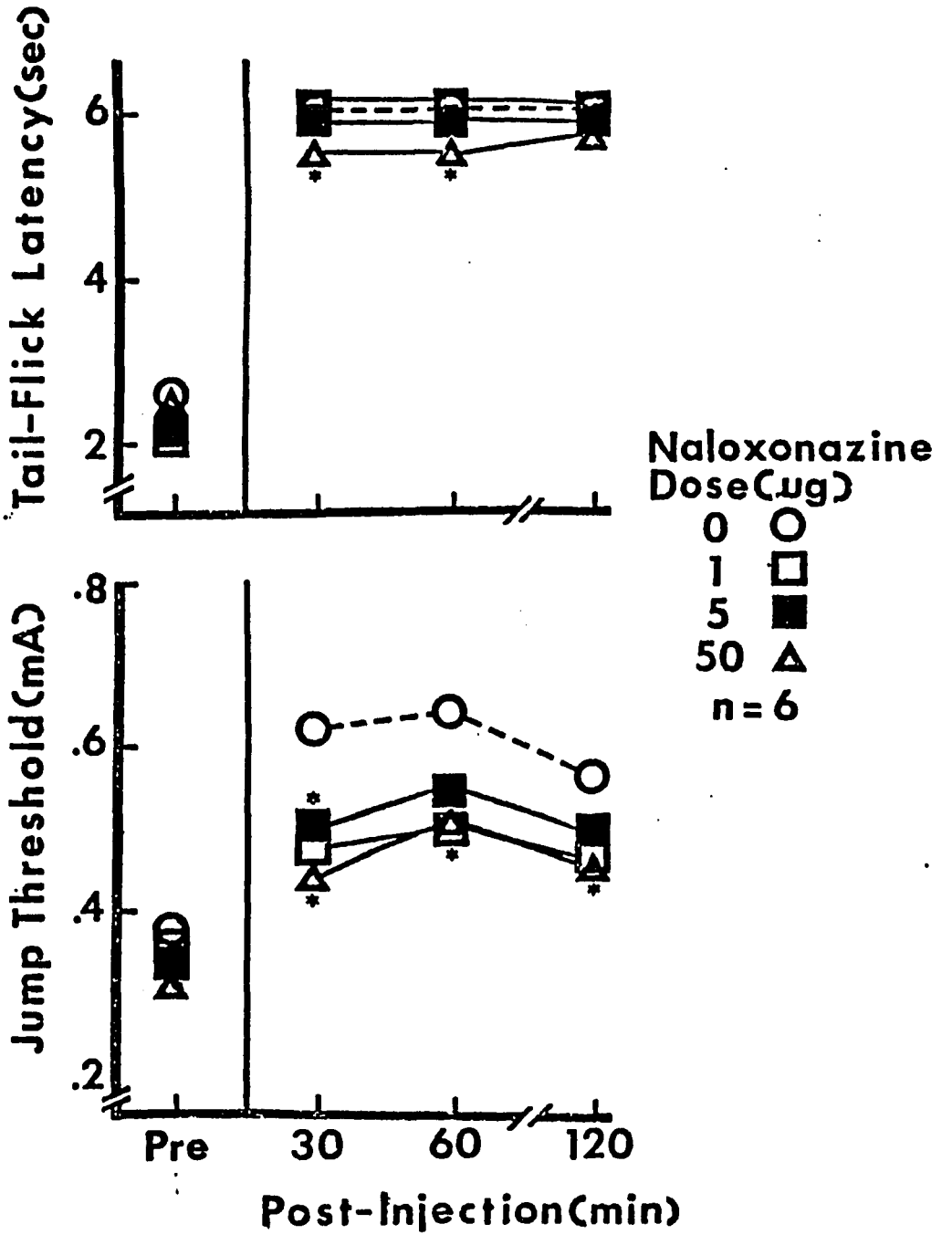

Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
Pre
30
60
120
Post-Injection(min)
Naloxonazine Dose(ug)
0
1
5
50
n=6

Figure 4. Reduction in morphine analgesia (5 mg/kg) 24 h following central pretreatment with naloxonazine. * indicates a significant difference from the vehicle-treated group, $p < .01$.

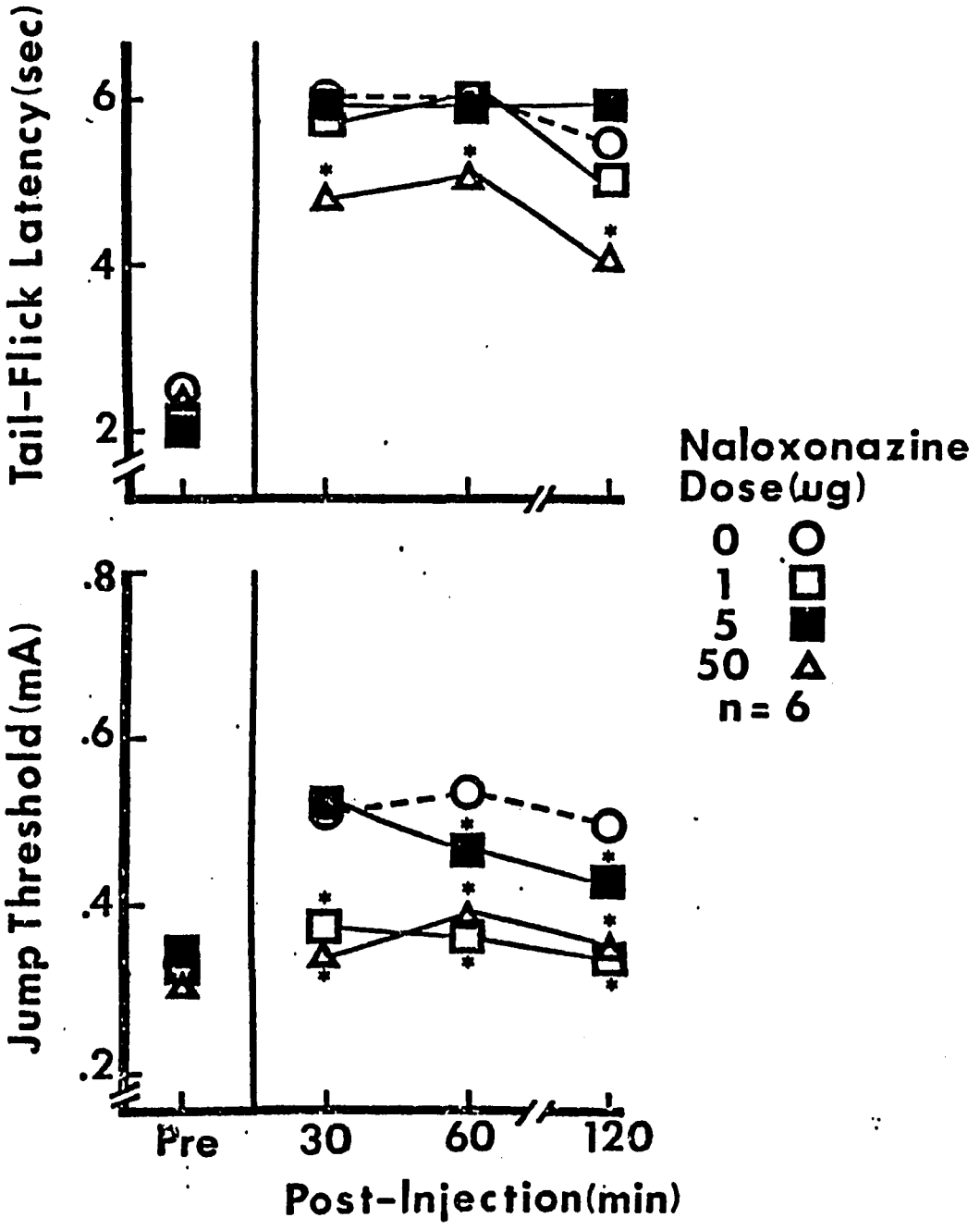

Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
Pre
30
60
120
Post-Injection(min)
Naloxonazine
Dose(ug)
0
1
5
50
n= 6

Figure 5. Reduction in morphine analgesia (2.5 mg/kg) 24 h following central pretreatment with naloxonazine. A significant difference from the vehicle-treated group is indicated by + ($p < .05$) or * ($p < .01$).

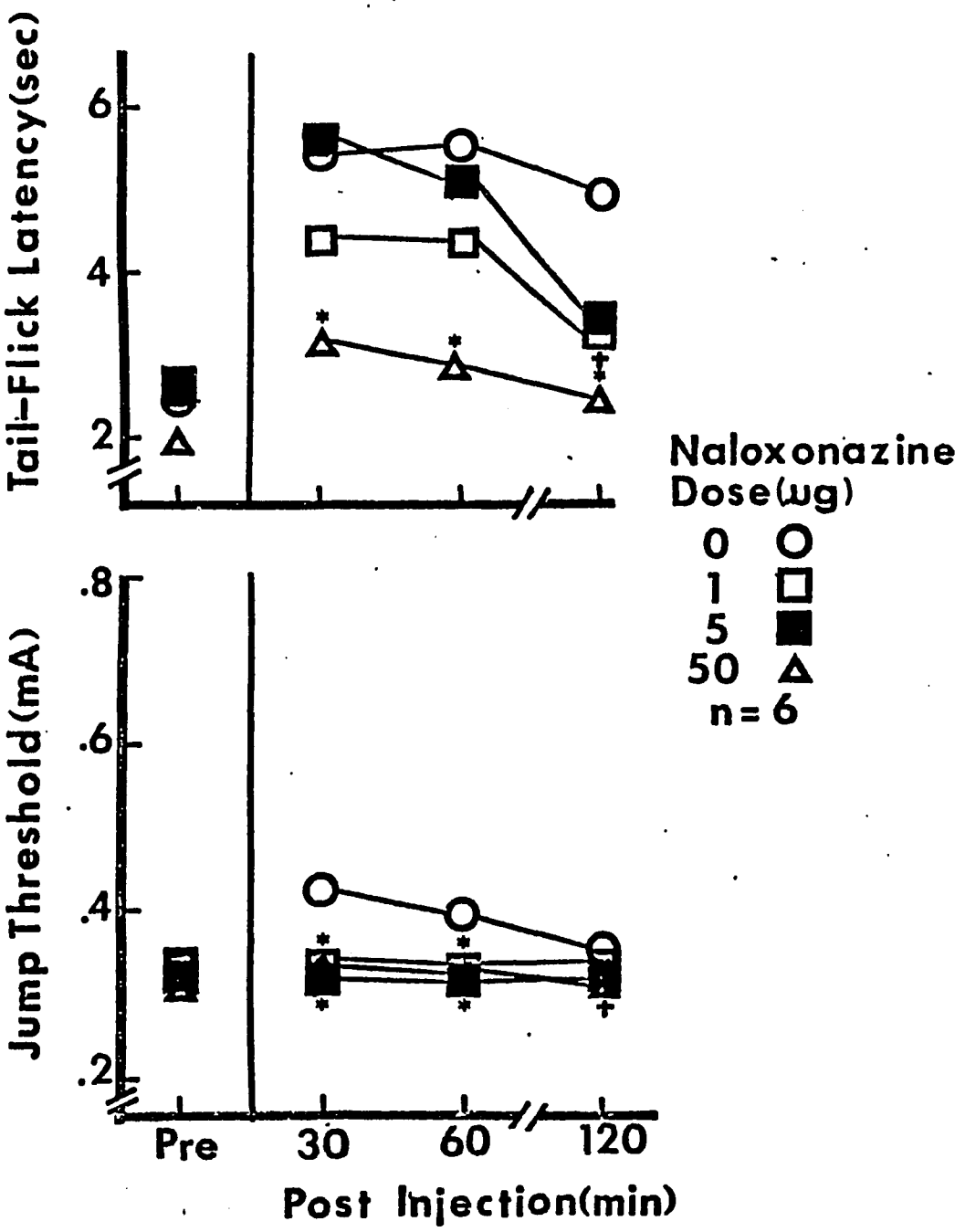
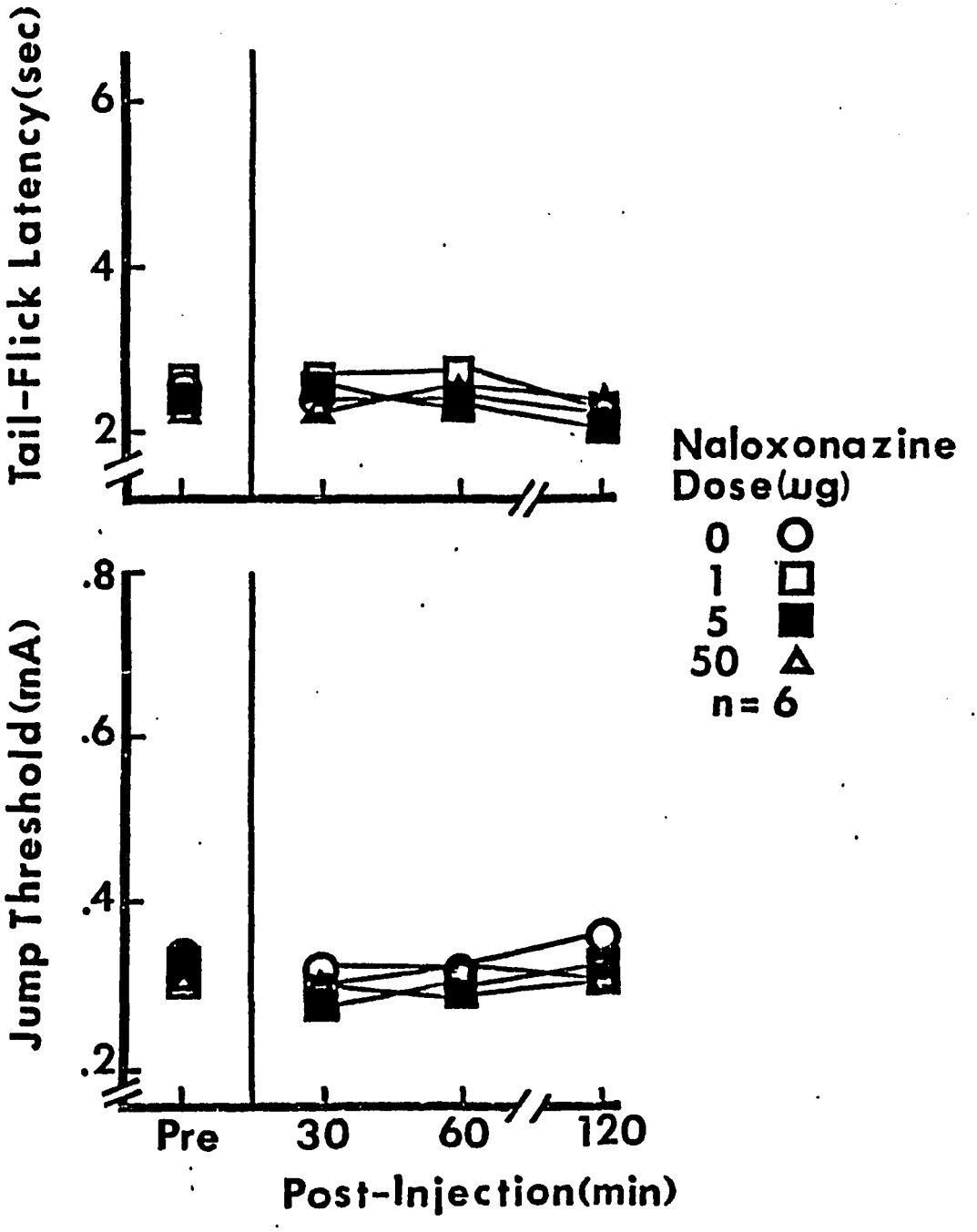

Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
Pre
30
60
120
Post Injection(min)
Naloxonazine
Dose(ug)
0
1
5
50
n=6

Figure 6. Failure of morphine (0.5 mg/kg) to induce analgesia on the tail-flick test or the jump test in naloxonazine or vehicle-treated rats.


Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
Pre
30
60
120
Post-Injection(min)
Naloxonazine
Dose(ug)
0
1
5
50
n= 6

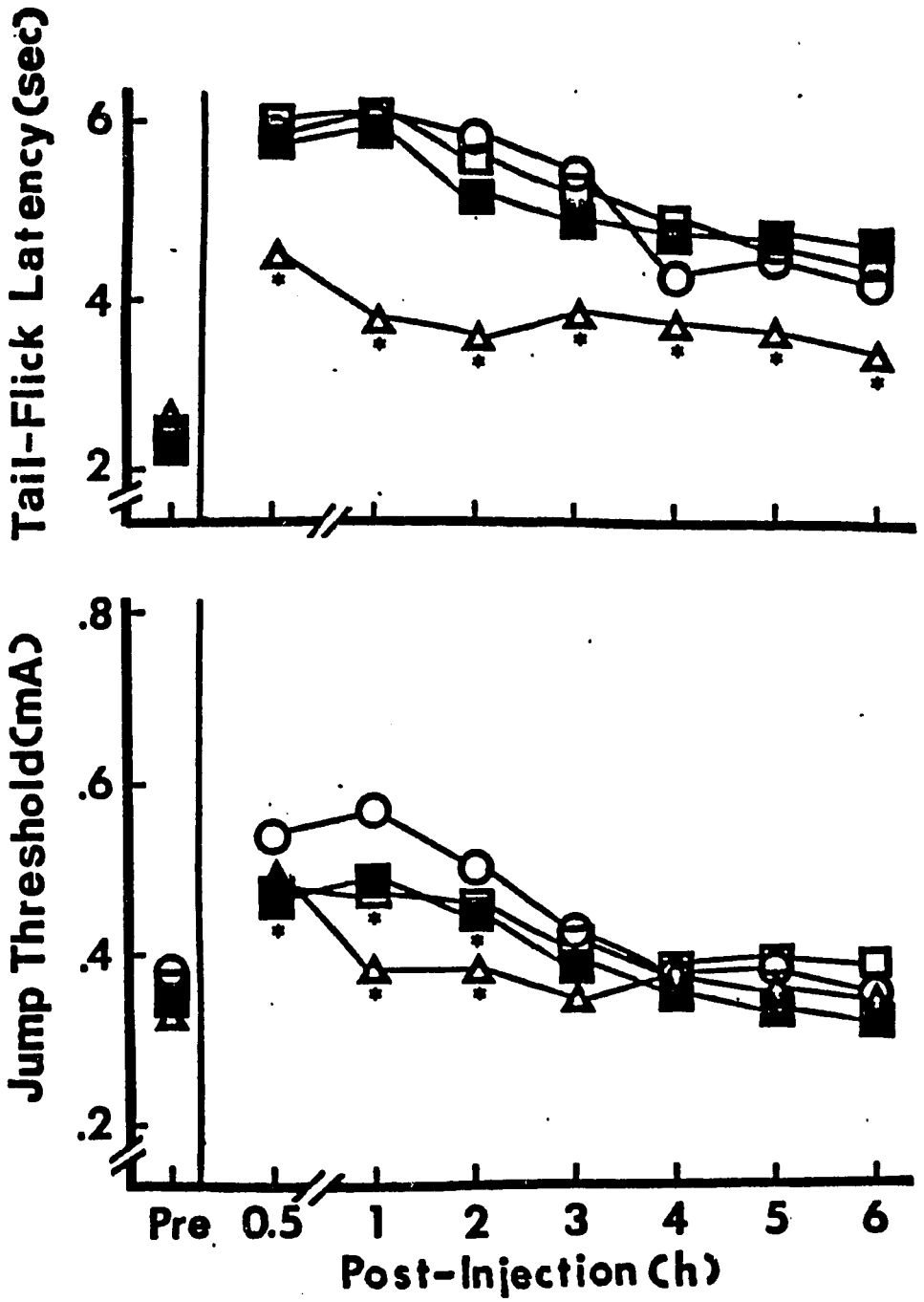
in jump thresholds were observed across the time course ($F(3,60) = 137.55$, $p < .001$) and for the interaction between doses and test times ($F(9,60) = 3.10$, $p < .004$), but not among doses ($F(3,20) = 1.76$). Further, while only the 50 ug dose of naloxonazine reduced analgesia measured on the tail-flick test induced by 5 and 10 mg/kg morphine was attenuated across the entire time course in rats pretreated with 1 and 50 ug naloxonazine, and at 30 and 60 min in rats pretreated with 5 ug naloxonazine (Dunnett comparisons). Significant differences in jump thresholds occurred among doses ($F(3,20) = 4.94$, $p < .01$), across test times ($F(3,60) = 102.45$, $p < .001$) and for the interaction between doses and test times ($F(9,60) = 2.41$, $p < .021$). Indeed, Figure 4 shows that analgesia measured across the time course by the 1 ug dose of naloxonazine, eliminated at 30 and 120 min and attenuated at 60 min by the 50 ug naloxonazine dose, and attenuated at 60 and 120 min following the 5 ug dose (Dunnett comparisons). Jump thresholds differed significantly among doses ($F(3,20) = 6.49$, $p < .003$), across test times ($F(3,60) = 34.49$, $p < .001$) and for the interaction between doses and test times ($F(9,60) = 5.90$, $p < .001$). Morphine analgesia measured on the tail-flick test induced by 2.5 mg/kg morphine was eliminated only by the 50 ug dose of naloxonazine, but Figure 5 shows that all three doses of naloxonazine eliminated analgesia on the jump test (Dunnett comparisons). Significant differences in jump thresholds were observed across the time course ($F(3,57) = 3.87$, $p < .014$), but jump thresholds failed to differ among doses ($F(3,19) = 2.07$) and there was no interaction between doses and test times ($F(9,57) = 1.42$). As on the tail-flick

test, the lowest morphine dose (0.5 mg/kg) failed to induce analgesia on the jump test for any group (Figure 6). Significant differences in jump thresholds occurred across test times ($F(3,60) = 6.30$, $p < .001$), but not among doses ($F(3,20) = 0.71$), nor for the interaction between doses and test times ($F(9,60) = 0.52$).

Insert Figure 7 here

Figure 7 shows that there was a significant reduction in morphine analgesia measured on the tail-flick test over a 6 h post-injection time course following naloxonazine pretreatment with 50 ug, but not 5 or 1 ug doses (Dunnett comparisons). Significant differences in tail-flick latencies occurred among the doses ($F(3,20) = 7.95$, $p < .001$), across the test times ($F(7,140) = 46.06$, $p < .001$) and approached significance for the interaction between doses and test times ($F(21,140) = 1.56$, $p < .067$). These data confirm our findings in experiment 1B. In contrast, all three naloxonazine doses attenuated morphine analgesia on the jump test. Significant differences in jump thresholds were observed across the time course ($F(7,140 = 40.38$, $p < .001$) and for the interaction between doses and test times ($F(21,140) = 2.48$, $p < .001$), but not among doses ($F(3,20) = 2.37$). Morphine analgesia was observed in all groups for up to 2 h following morphine administration, but these analgesic responses were significantly attenuated in all naloxonazine-pretreated groups (Dunnett comparisons). Furthermore, although the 1 and 5 ug naloxonazine doses reduced morphine analgesia similarly, the effect was

Figure 7. Dose-dependent reductions in morphine analgesia (5 mg/kg) on the tail-flick test and jump test 24 h following central pretreatment with naloxonazine. The open circles represent the vehicle-treated group. Naloxonazine-treated groups are indicated by the open squares (1 ug), solid squares (5 ug) and open triangles (50 ug). * indicates a significant difference from vehicle-treated rats, $p < .01$).


Tail-Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
Pre
0.5
1
2
3
4
5
6
Post-Injection (h)

more pronounced following pretreatment with the 50 ug naloxonazine dose.

EXPERIMENT 1C: Central naloxone and morphine analgesia.

Rationale: Both central and systemic administration of naloxone eliminate or attenuate morphine analgesia with the magnitude of the reversal dependent upon the naloxone and morphine doses employed. For naloxone to be maximally effective, the interval between naloxone and morphine injections must typically be less than 30 min. In contrast, both central and systemic administration of naloxazone and naloxonazine attenuate or eliminate morphine analgesia even when the injection interval between these agents and morphine is 24 h. The present experiment is a control experiment designed to demonstrate naloxone's failure to possess long-lasting effects upon morphine analgesia even when injected centrally and thereby confirm naloxone's short duration of action.

METHOD

Two groups of six cannulated rats received icv injections of either vehicle or 50 ug of naloxone respectively. Twenty-four h later, tail-flick latencies and jump thresholds were assessed immediately prior to and 0.5, 1 and 2 h following a 10 mg/kg subcutaneous dose of morphine.

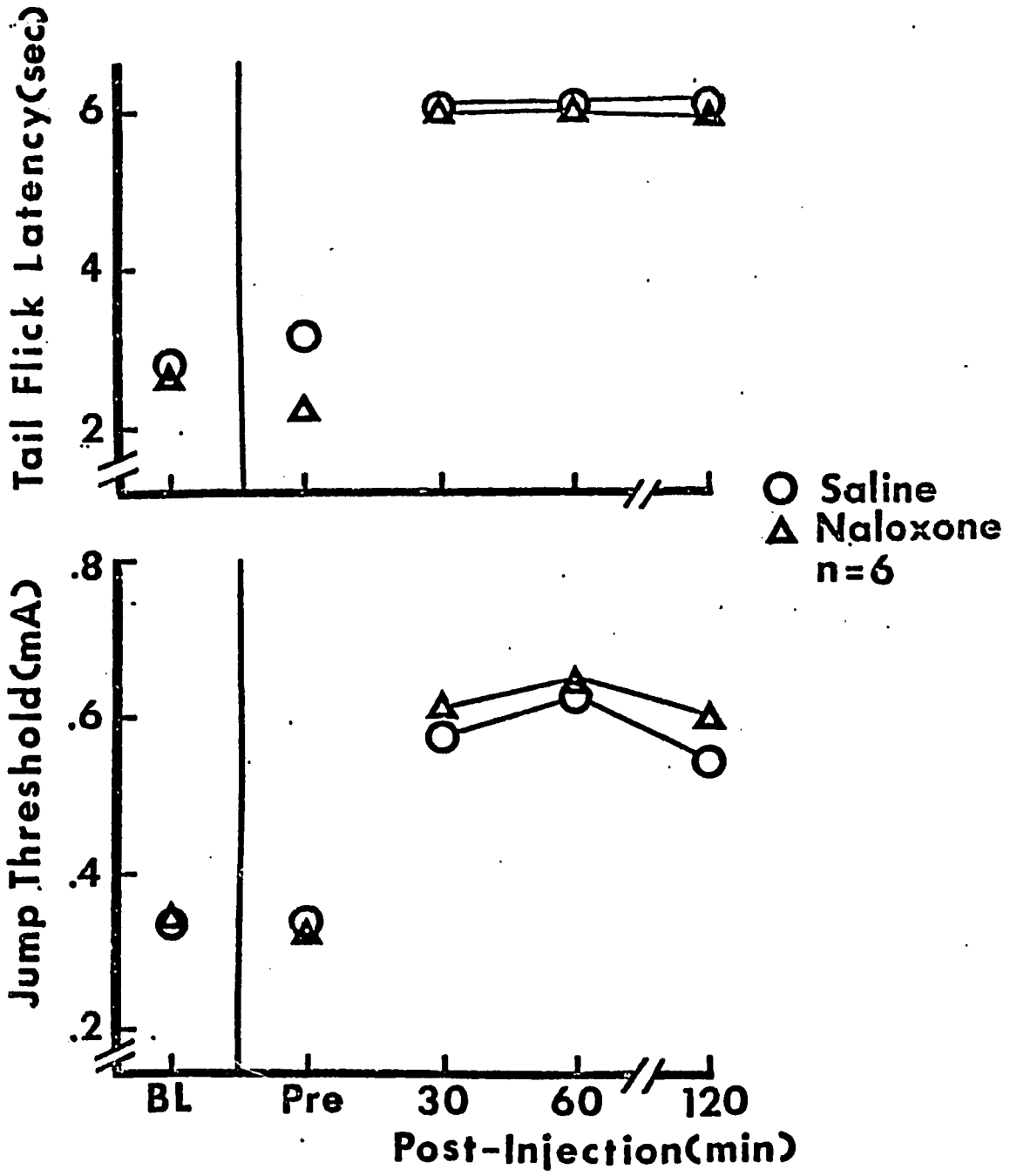
Insert Figure 8 here

Figure 8 shows that central pretreatment with a 50 ug dose of naloxone failed to alter morphine analgesia measured on either the tail-flick or jump test after 24 h. While significant increases in tail-flick latencies and jump thresholds were observed across test times (tail-flick: ($F(7,77) = 656.64$, $p < .001$); jump: $F(7,77) = 125.36$, $p < .001$), significant differences failed to occur among dose (tail-flick: $F(1,11) = 1.96$; jump: $F(1,11) = 0.49$) or for the interaction between doses and test times (tail-flick: $F(7,77) = 1.09$; jump: $F(7,77) = 0.75$).

EXPERIMENT 1D: Intravenous naloxonazine and morphine analgesia.

Rationale: Both central and systemic administration of opiate antagonists such as naloxone and naloxazone are effective in attenuating or eliminating morphine analgesia. Intravenous pretreatment with naloxonazine reduces morphine analgesia in mice on the tail-flick (Ling et al., 1983). The results of Experiment 1B indicated that central pretreatment with naloxonazine reduced morphine analgesia on the tail-flick test only at very low morphine doses. Two possibilities may explain these results: injection route differences or species differences. The present experiment was a systematic replication of the previous study (Ling et al., 1983) except that rats

Figure 8. Lack of alterations in morphine analgesia (10 mg/kg) 24 h following central pretreatment with naloxone (50 ug).


Tail Flick Latency(sec)
6
4
2
Jump Threshold(mA)
.8
.6
.4
.2
BL
Pre
30
60
120
Post-Injection(min)
O Saline
Δ Naloxone
n=6

were used as subjects. A comparison of route effects could tnus be made without the possible confounding variable of species differences.

METHOD

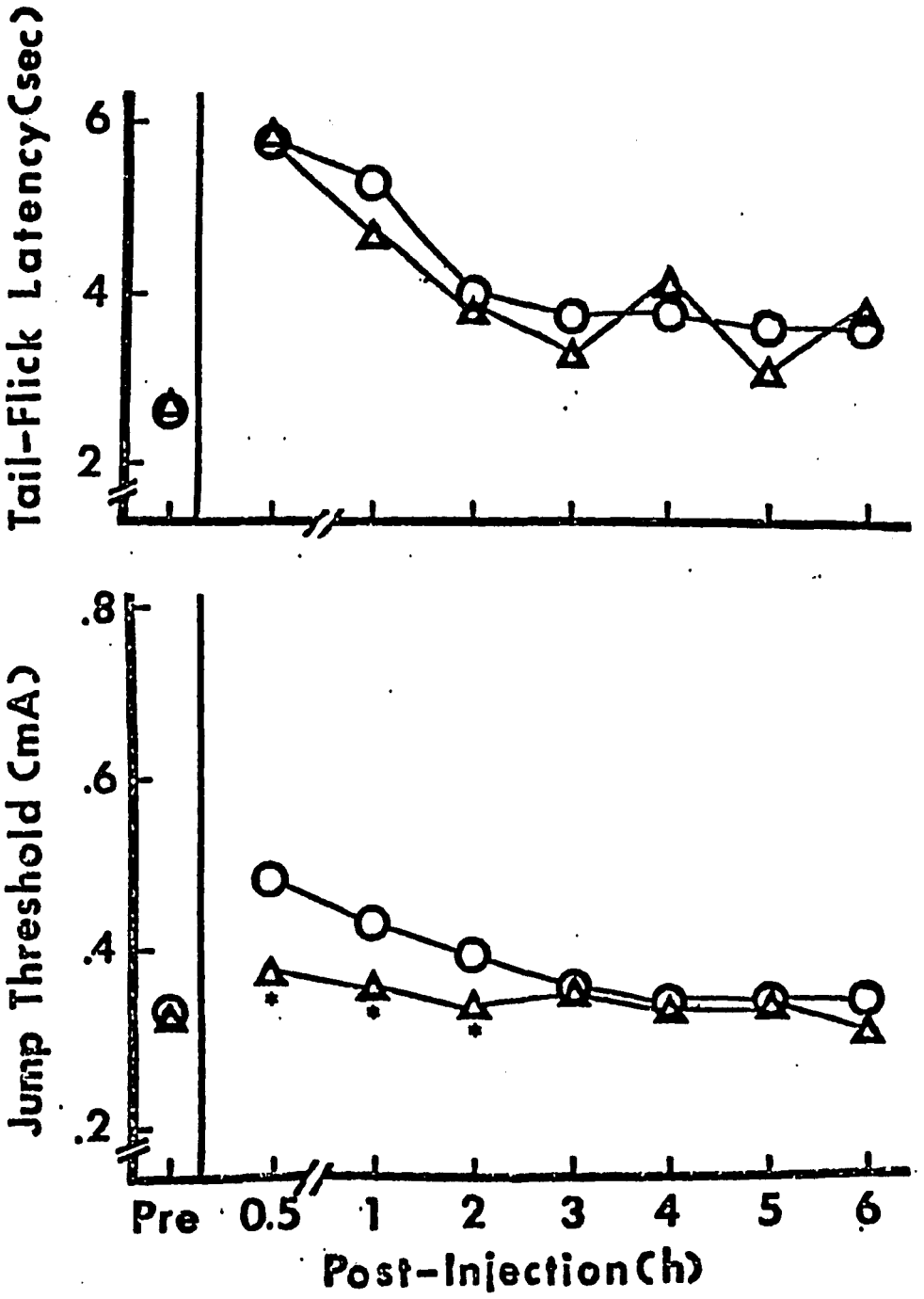
Two groups of six rats were used. As in a study by Ling et al. (1983), twenty-four h following intravenous pretreatment with either vehicle or 10 mg/kg naloxonazine, all animals received an intravenous injection of morphine (3 mg/kg). Tail-flick latencies and jump thresholds were assessed just prior to and at 0.5, 1, 2, 3, 4, 5 and 6 h following morphine administration.

RESULTS

Insert Figure 9 here

Figure 9 shows the alterations in analgesia measured on the tail-flick test and jump tests induced by 3 mg/kg morphine iv 24 h following iv naloxonazine pretreatment. There were significant differences in tail-flick latencies occurred across the test times ($F(7,70) = 15.19$, $p < .001$), but there were no significant differences among treatments ($F(1,10) = 0.27$) nor for the interaction between treatment and test time ($F(7,70) = 0.55$. Tail-flick latencies of vehicle-treated animals were significantly elevated across the entire 6 h time course, but naloxonazine-treated animals failed to display analgesia at 3 and at 5 h following the injection (Dunnett

Figure 9. Elimination of morphine analgesia (3 mg/kg, iv) on the jump test without alterations in morphine analgesia on the tail-flick test 24 h following intravenous pretreatment with naloxonazine 10 mg/kg. The open circles represent the vehicle-treated group and the open triangles represent the naloxonazine-treated group. * refers to a significant difference from the vehicle-treated group, $p < .01$.


Tail-Flick Latency(sec)
6
4
2
Jump Threshold (mA)
.8
.6
.4
.2
Pre
0.5
1
2
3
4
5
6
Post-Injection(h)

comparisons). However, the reduction in analgesia in naloxonazine pretreated rats failed to differ significantly from the vehicle-treated animals. Figure 9 also shows that intravenous naloxonazine eliminated the morphine analgesia seen on the jump test. Significant differences in jump thresholds were observed across test times ($F(7,70) = 11.44$, $p < .001$) and for the interaction between treatment and test times ($F(7,70) = 2.83$, $p < .01$) but not among treatments ($F(1,10) = 1.53$).

DISCUSSION

These studies examined the effects of naloxonazine upon basal pain thresholds and analgesia induced by morphine 24 h subsequently and showed that a) intracerebroventricular administration of naloxonazine failed to alter either baseline tail-flick latencies or jump thresholds for up to 24 h following its administration; b) central pretreatment with naloxonazine, but not naloxone, attenuated or eliminated morphine analgesia in a dose-dependent and test-dependent manner measured on the tail-flick and jump tests; and c) intravenous naloxonazine pretreatment attenuated morphine analgesia measured on the jump test but not the tail-flick test.

The results of the present study that pretreatment with naloxonazine did not alter basal tail-flick latencies or jump thresholds (Experiment 1A) are in agreement with previous studies employing naloxone, and suggest that tonic influences play at best a minor role in the activation of opioid systems in analgesia (Goldstein et al., 1976; North, 1977; Weisenfeld and Hallin, 1983). It therefore appears that Mul opioid binding sites are activated in a phasic manner in producing opiate analgesia. However, it should be noted that the present study employed naloxonazine doses sufficient to produce attenuations of morphine analgesia (see following discussion). It is possible that a MU1-mediated tonic system may be blocked by higher naloxonazine doses. Furthermore, other opiate receptor sub-types may provide tonic influences on pain perception. For example, since naloxone does not discriminate between MU1 and MU2 sites, one cannot

rule out the possibility that MU2 sites modulate the reported hyperalgesia following naloxone administration (Carmody et al., 1979; Jacob et al., 1974; Woolf, 1980b). Opiate antagonists specific for MU2 and other opiate receptor sub-types need to be developed in order to address this issue.

The degree to which central administration of naloxonazine doses of 1, 5 and 50 ug attenuate analgesia induced by a range of morphine doses (15-0.5 mg/kg) measured on the tail-flick and jump tests 24 h later (Experiment 1B) depended upon naloxonazine and morphine doses as well as the nociceptive test. In summary, central pretreatment with 50 ug of naloxonazine was effective in significantly reducing morphine analgesia at 15 mg/kg on the jump test and at 5 mg/kg on the tail-flick test, a three-fold difference between tests. The 50 ug dose of naloxonazine eliminated morphine analgesia at 5 mg/kg on the jump test and at 2.5 mg/kg on the tail-flick test, a two-fold difference between tests. In contrast, following a 1 ug dose of naloxonazine, reductions in morphine analgesia are observed at 15 mg/kg on the jump test and at 2.5 mg/kg on the tail-flick test, a six-fold difference between tests. Elimination of morphine (5 mg/kg) analgesia was observed following a 1 ug dose of naloxonazine on the jump test, but analgesia was never completely eliminated on the tail-flick test at this dose. However, since a cut-off value of 6 sec was employed in the tail-flick test, it is possible that naloxonazine's effects were, in part, influenced by a ceiling effect on this nociceptive measure. Interestingly, central pretreatment with naloxazone 50 ug decreases morphine analgesia (5 mg/kg) by 49% on the

jump test (Kirchgessner et al., 1982). Naloxonazine eliminated morphine analgesia on this test following the same drug and test time parameters. Although naloxazone and naloxonazine have identical biochemical effects, these data demonstrate behaviorally the superior potency of naloxonazine to naloxazone at equimolar doses.

In investigating the dose-response relationships between naloxonazine and morphine, it appeared initially that naloxonazine's dose-response curve was not completely uniform since the 1 ug naloxonazine dose appeared more effective in reducing morphine analgesia than the 5 ug dose (see Figures 2-5). However, for the last four groups of animals who were tested for morphine analgesia over an extended time course, the 1 and 5 ug naloxonazine doses failed to differ from each other and were less effective than the 50 ug dose (Figure 7). It is therefore difficult to make any definitive statements regarding the relationship between the 1 and 5 ug doses of naloxonazine. Further studies are in progress re-evaluating naloxonazine's effects over a larger dose range. It should be noted that we were primarily interested in the dose-response relationships between naloxonazine and morphine analgesia and therefore analyzed the effects of naloxonazine upon morphine analgesia. However, one could examine the influence of morphine on naloxonazine's effects in analgesic and other behaviors also.

Intravenous naloxonazine pretreatment (10 mg/kg) also resulted in test specific effects, with the jump test appearing more sensitive than the tail-flick test to naloxonazine's effects. Morphine (3 mg/kg, iv) analgesia was eliminated on the jump test but was

unaffected on the tail-flick test (Figure 9). Since less than 0.1% of intravenous naloxonazine crosses the blood-brain barrier (G.W. Pasternak, personal communication), the intravenous naloxonazine dose and the icv doses employed in the present study are comparable in concentration reaching the central nervous system. It is therefore surprising that intravenous naloxonazine failed to alter morphine analgesia (3 mg/kg, iv) on the tail-flick test. One possibility is that the icv naloxonazine and the intravenous naloxonazine which enters the central nervous system may be differentially available to opiate receptors. This may be due to differences in the diffusion of naloxonazine into tissue from the ventricle or the bloodstream. The rate and extent of diffusion of naloxonazine from the ventricle is unknown but may be a factor in determining the duration and extent of icv effects. Further studies using autoradiography to determine access of different injection routes may provide a more definitive answer to this point.

One reason for the difference between results using different measures of analgesia is that the analgesic mechanism for each pain test may be different. To illustrate this point, some of the biochemical and anatomical mechanisms mediating opiate analgesia in general will be reviewed, indicating that these mechanisms can be dissociated according to the particular type of nociceptive stimulus employed. The opioid-mediated pain inhibitory system proposed by Fields and Basbaum (1978) originates in the midbrain PAG, synapses in the NRM and inhibits dorsal horn nociceptive neurons through its serotonergic projection. That serotonin is integral for the

expression of opiate analgesia (see reviews: Mayer and Price, 1976; Messing and Lytle, 1977; Yaksh and Rudy, 1978) is supported by the fact that opiate analgesia is reduced following lesions placed in the NRM (Proudfit and Anderson, 1975; Samanin et al., 1973) or the DLF (Basbaum, Marley, O'Keefe, and Clanton, 1977) and following serotonin depletion by 5,6-dihydroxytryptamine (5,6 DHT) (Vogt, 1974) or p-chlorophenylalanine (Tenen, 1968). In addition, noradrenergic neurons may modulate the antinociceptive effects of opiates. Biochemical and histochemical studies have revealed noradrenergic terminals in the NRM (Fuxe, 1965; Levitt and Moore, 1979; Saavedra, Grobecker, and Zinin, 1976), where iontophoretic application of norepinephrine inhibits NRM neuronal activity (Wessendorf, Proudfit, and Anderson, 1981). Administration of alpha-adrenergic antagonists such as phentolamine into the NRM produce analgesia (Hammond, Levy, and Proudfit, 1980a). However, descending noradrenergic pathways into the spinal cord (Nygren and Olsen, 1977) are differentially involved in antinociception since intrathecal administration of norepinephrine produces analgesia (Reddy and Yaksh, 1980; Reddy, Manderdrut, and Yaksh, 1980). The supraspinal noradrenergic system interacts with spinal noradrenergic and serotonergic systems since the analgesia following phentolamine administered into the NRM is reversed by intrathecal application of either phentolamine (Sagen and Proudfit, 1981) or methysergide (Hammond, Levy, and Proudfit, 1980b). These spinal noradrenergic and serotonergic systems also alter opiate analgesia. Opiates have been shown to produce their antinociceptive effects through supraspinal and spinal mechanisms (see review: Yaksh

and Rudy, 1977). Analgesic responses on the tail-flick and hot plate tests following systemic morphine administration are attenuated either by icv or intrathecal naloxone administration (Yeung and Rudy, 1980a). Furthermore, the analgesic effects of icv and intrathecal morphine injections potentiate each other (Yeung and Rudy, 1980a). Collectively, these studies demonstrate that opiate analgesia is mediated through several neurotransmitter systems at both spinal and supraspinal anatomical loci.

Given both spinal and supraspinal mechanisms of opiate action, it has been demonstrated that the nociceptive stimulus employed is a critical factor in determining which mechanisms will be activated. For instance, lesions placed in either the NRM or the PAG attenuate morphine analgesia on the tail-flick test, but fail to do so on the formalin test (Abbott, Melzack, and Samuel, 1982). Furthermore, lesions placed in the pontine tegmentum or median raphe potentiate morphine analgesia on the tail-flick and formalin tests respectively (Abbott and Melzack, 1982). Recent evidence suggests that the involvement of spinal serotonergic and noradrenergic systems in morphine analgesia can be dissociated across pain tests. Intrathecal administration of the catecholamine neurotoxin 6-hydroxydopamine, but not the serotonin neurotoxin (5,6 DHT), reduces morphine analgesia on the tail-pinch test (Kuraishi, Harada, Aratani, Satoh and Takagi, 1983). In contrast, intrathecal administration of 5,6 DHT attenuates morphine analgesia on the hot plate test, but not on the tail-flick or tail-pinch tests. Intrathecal administration of norepinephrine is more potent than

serotonin in producing analgesia on the tail-pinch test (Kuraishi et al., 1983)J, suggesting a primary role for noradrenergic mechanisms in morphine's analgesic response to mechanical stimulation and a primary role for serotonergic mechanisms in morphine's analgesic response to thermal stimuli.

Specific spinal opiate receptor sub-types also appear to be involved in the selective modulation of opiate analgesia as a function of the pain stimulus employed. Intrathecal administration of mu-receptor (morphine) and delta-receptor (enkephalin), but not kappa-receptor (ethylketocyclazocine, bremazocine or U50488H) agonists produce dose-dependent increases in hot plate and tail-flick latencies (Schmauss and Yaksh, 1984). In contrast, the mu and kappa agonists, but not delta agonists, produce analgesia on the writhing test. These data suggeste that the mu-receptor is involved in the expression of opiate analgesia across many pain stimuli while the delta and kappa receptors mediate opiate analgesia in response to noxious thermal or visceral stimulation respectively. Furthermore, beta-funaltrexamine (B-FNA), a short-lasting kappa-receptor agonist and long-lasting mu-receptor antagonist, induces analgesia on the writhing, but not the tail flick test (Ward, Portoghese, and Takemori, 1982).

The above studies demonstrate that opiate analgesia may be the result of a complex interaction between opiate receptor sub-types, spinal and supraspinal peptide and transmitter systems, and the type of nociceptive stimulus. Since naloxonazine is more effective in reducing morphine analgesia on the jump test than on the spinally-mediated tail-flick test, it would appear that it acts

primarily upon supraspinal mechanisms by interacting with MU1 opiate binding sites (Ling and Pasternak, 1983). Therefore, opiate analgesia as measured by the jump test, may involve supraspinal mechanisms while analgesia on the tail-flick test may be the result of a complex interaction between opiate receptor sub-types, spinal and supraspinal peptide and transmitter systems, and the type of nociceptive stimulus. Since naloxonazine is more effective in reducing morphine analgesia on the jump test than on the spinally-mediated tail-flick test, it would appear that it acts primarily upon supraspinal mechanisms by interacting with MU1 opiate binding sites (Ling and Pasternak, 1983). Therefore, opiate analgesia as measured by the jump test, may involve supraspinal mechanisms while analgesia on the tail-flick test may be mediated by both spinal and supraspinal mechanisms (Yeung and Rudy, 1980a, b).

It has been shown biochemically that naloxonazine exerts its effects by binding to the high affinity binding site (MU1) of all opiate receptor sub-types (Hahn and Pasternak, 1982). Naloxone, although it binds to both high and low affinity binding sites, does not compete equally well for all receptor sub-types (Lord et al., 1977). However, biochemical studies are done in vitro and it is possible that naloxonazine may be having its in vivo effects by a conversion into naloxone. Vehicle-treated animals in Experiments 1B and 1C displayed a similar analgesic response following a morphine dose of 10 mg/kg. However, central pretreatment with either naloxone or naloxonazine doses of 50 ug produced clearly different effects upon morphine analgesia (Figures 3 and 8). Since naloxone, unlike

naloxonazine, failed to alter morphine analgesia 24 h later, this supports the contention that naloxonazine's effects are due to its long-term ability to bind to MU1 opiate binding sites. Since naloxonazine binds equally well to this binding site of all opiate receptor sub-types, it is not yet known which receptor type is most important in opiate analgesia. One hypothesis is that the MU1 sites may actually represent a distinct sub-class of receptors which mediate opiate analgesia (Pasternak, 1982). Furthermore, the precise localization of these MU1 binding sites relative to MU2 and other opioid receptor binding sites is still unclear. On the basis of lesion and microinjection studies, the PAG and NRM are possible anatomical loci which may contain the MU1 binding sites required for the full expression of morphine analgesia.

EXPERIMENT 2: Naloxonazine and ingestive processes.

EXPERIMENT 2A: Intravenous naloxonazine and free feeding.

Rationale: Acute administration of naloxone decreases nocturnal feeding in rats (Holtzman, 1974; Brown and Holtzman, 1979; Cooper, 1980; Brands et al., 1979). Chronic infusion of naloxone also attenuates free feeding (Jaloweic et al., 1981). The present experiment examined whether intravenous administration of naloxonazine would reduce feeding over 24 h following the injection.

METHOD

Thirty-two rats were implanted with iv catheters and allowed a two day recovery period. Food intake over 24 h was determined by placing a pre-weighed food bin of Purina Rat Chow into the rat's wire mesh cage at 1-3 h into the light cycle, and weighing the bin 24 h thereafter. The food spillage was collected, weighed and accounted for in intake measures. Following determination of baseline 24 h food intakes over a two day period, animals were matched into two equal groups according to their 24 h food intake and were given an iv injection of either naloxonazine (10 mg/2 ml/kg body wt) or vehicle. Food consumption was assessed 24 h thereafter.

RESULTS

Insert Table 1 here

TABLE 1. Reductions in mean food intake (Standard Error of the Mean: SEM) over a 24 h period following intravenous naloxonazine (10 mg/kg) pretreatment.

	Food Intake (grams)	
Group	Baseline	Post-injection
Vehicle (n=16)	20.2 (1.0)	18.1 (0.7)*
Naloxonazine (n=16) 10 mg/kg, iv	21.7 (0.7)	14.7 (0.9)*+

Note 1: * indicates a significant difference from baseline and + indicates a significant difference from vehicle (Dunnett comparison, $p < .01$).

Table 1 shows that there was a significant reduction in free feeding 24 h following iv administration of 10 mg/kg of naloxonazine. Significant differences between the groups in baseline food intake failed to occur ($F(1,30) = 1.02$), but significant differences were observed between baseline and injection conditions ($F(1,30) = 43.97$, $p < .001$) and for the interaction between groups and conditions ($F(1,30) = 12.96$, $p < .001$). Decreases in food intake were observed in both vehicle-treated and naloxonazine-treated animals; however, the magnitude of hypophagia was significantly greater in the naloxonazine-treated group (68% of baseline) as compared to the vehicle-treated group (90% of baseline) (Dunnett comparisons).

EXPERIMENT 2B: Acute naloxonazine or naloxone administration and deprivation-induced food intake.

Rationale: The opiate antagonist naloxone has been shown to produce its greatest anorectic effects in rats deprived of food (Holtzman, 1974; Frenk and Rogers, 1979; Brown and Holtzman, 1979). However, it is short-acting and, to produce its anorectic effect, it must be administered just prior to the reintroduction of food. The present experiment evaluated whether iv administration of either naloxonazine or naloxone would reduce intake after a 24 h deprivation period in a similar fashion to that of systemic naloxone administered just prior to the reintroduction of food.

METHOD

Forty-nine rats were divided into five groups. Three groups received iv injections of either vehicle or naloxonazine at doses of 10 and 20 mg/kg respectively, and were then deprived of food for 24 h. The fourth and fifth groups were deprive of food for 24 h and then received 10 mg/kg naloxone either intravenously 10 min prior to food reintroduction or subcutaneously immediately prior to food reintroduction. Food intake was assessed two and 24 h thereafter.

RESULTS

Insert Tables 2 and 3 here

Significant differences in food intake at 2 h following food reinstatement occurred among the groups ($F(5,43) = 3.41$, $p < .02$). Table 2 indicates that there was a significant reduction in intake at 2 h following reintroduction of food in animals pretreated systemically with either naloxonazine or naloxone (Dunn comparisons). Administration of naloxonazine at doses of 10 and 20 mg/kg administered at the beginning of the 24 h deprivation period significantly attenuated intake 2 h after food reintroduction (67% and 66% of control respectively). In contrast, naloxone administration at this same time interval failed to alter deprivation-induced intake. However, if naloxone was administered within 15 min prior to the reintroduction of food by either intravenous or subcutaneous routes of

TABLE 2. Reduction in 24 h deprivation-induced food intake (grams: SEM) following systemic naloxone or naloxonazine pretreatment at 2 h following food reinstatement.

-	Drug/feeding interval	Food intake over 2 h	Difference in feeding from vehicle
Vehicle (10)	24 h	8.2 g (0.6)	
Naloxonazine (7) 10 mg/kg, iv	24 h	5.5 g (1.2)+	-33%
Naloxonazine (8) 20 mg/kg, iv	24 h	5.4 g (1.4)+	-34%
Naloxone (8) 10 mg/kg, iv	24 h	8.9 g (0.8)	
Naloxone (10) 10 mg/kg, iv	10 min	4.8 g (0.9)+	-42%
Naloxone (6) 10 mg/kg, sc	0 min	5.0 g (0.8)+	-39%

Note 1. + indicates a significant difference from vehicle (Dunnett comparison, $p < .05$).

TABLE 3. Failure of systemic naloxone or naloxonazine pretreatment to alter deprivation-induced food intake (grams) 24 h following food reinstatement.

Group (n)	Drug/feeding interval	Food intake over 24 h
Vehicle (10)	24 h	32.6
Naloxonazine (7) 10 mg/kg, iv	24 h	27.3
Naloxonazine (8) 20 mg/kg, iv	24 h	22.9
Naloxone (8) 10 mg/kg, iv	24 h	33.7
Naloxone (10) 10 mg/kg, iv	10 min	29.7
Naloxone (6) 10 mg/kg, sc	0 min	25.8

administration, it significantly reduced intake (59% and 61% of control respectively). Interestingly, the magnitude of intake inhibition failed to differ between naloxonazine administered 24 h prior and naloxone administered 15 min prior to food reintroduction. However, while naloxonazine and naloxone inhibited intake 2 h following reintroduction of food, Table 3 shows that significant effects were not present 24 h after food reintroduction ($F(5,43) = 1.74$).

EXPERIMENT 2C: Acute naloxonazine or naloxone administration and 2-DG-induced hyperphagia.

Rationale: 2-DG induces glucoprivation (Wick et al., 1957) and hyperphagia (Smith and Epstein, 1969) which is attenuated by naloxone (Lowy et al., 1980; Sewell and Jawaharlal, 1980). Maximal 2-DG hyperphagia occurs within 4 h following 2-DG administration and can be attenuated by naloxone given just prior to the 2-DG. To assess naloxonazine's long-term anorectic effects, the present study compared pretreatment with naloxonazine 24 h prior to the 2-DG administration upon 2-DG hyperphagia with naloxone administered just prior to the 2-DG injection.

METHOD

Three groups of eight rats were used. The first two groups received intravenous vehicle or 10 mg/kg of naloxonazine 24 h prior to

intraperitoneal 2-DG (400 mg/kg). The third group received 10 mg/kg of naloxone subcutaneously just prior to 2-DG administration, thus replicating previous work (Lowy et al., 1980; Sewell and Jawaharlal, 1980). Food consumption was assessed 2 and 4 h following the 2-DG injection.

RESULTS

Insert Table 4 here

Significant differences in food intake were observed among the groups at 2 h ($F(2,21 = 15.72$, $p < .001$) and at 4 h ($F(2,21 = 9.87$, $p < .001$) following 2-DG administration. Table 4 shows that immediate pretreatment with naloxone significantly attenuated food intake at 2 and 4 h following 2-DG administration (Dunnett comparisons). In contrast, long-term pretreatment with naloxonazine failed to alter intake 2 h following 2-DG and significantly increased food intake 2 h later. These results are in agreement with previous findings (Lowy et al., 1980; Sewell and Jawaharlal, 1980) that naloxone, when administered just prior to 2-DG, attenuates the 2-DG induced hyperphagic response, but demonstrates a clear dissociation between naloxone's short-term and naloxonazine's long-term effects on this measure.

TABLE 4. Alterations in 2-DG (400 mg/kg) hyperphagia following systemic naloxone or naloxonazine pretreatment.

	Post-2-DG (h) Food Intake (grams: SEM)	
Group (n)	2	4
Vehicle (8)	4.3 (0.7)	6.1 (0.8)
Naloxonazine (8) 10 mg/kg, iv	5.3 (0.7)	8.5 (1.0)+
Naloxone (8) 10 mg/kg, sc	0.7 (0.3)++	3.3 (0.7)++

Note 1. Significant difference from vehicle (Dunnett comparisons, + $p < .05$; ++ $p < .01$).

EXPERIMENT 2D: Central administration of naloxonazine and free feeding.

Rationale: Central administration of either morphine or endogenous opioid peptides elicits feeding which is reversed by naloxone (McKay et al., 1979; Morley and Levine, 1982; Leibowitz and Hor, 1980; McLean and Hobel, 1980). However, the long-term effects of centrally-adminsitered opiate antagonists upon ingestion has not been investigated. The purpose of the present experiment was to examine the effects of centrally-applied naloxonazine upon free feeding and to determine if these effects are similar to those produced following iv administration.

METHOD

Twenty-four cannulated rats were divided into four groups. Since these animals were the same as those used in Experiment 1A, they were matched according to their baseline tail-flick latencies and jump thresholds. Each animal received icv vehicle or naloxonazine at a dose of either 1, 5, or 5 ug. Food consumption was measured over 24 h following the injection.

RESULTS

Insert Table 5 here

TABLE 5. Failure of central administration of naloxonazine to alter free feeding over 24 h.

Group	Food Intake (grams: SEM)
Vehicle	16.7 (1.4)
Naloxonazine (1 ug)	11.8 (1.9)
Naloxonazine (5 ug)	14.9 (1.5)
Naloxonazine (50 ug)	17.6 (1.5)

Free feeding was not altered by any of the naloxonazine doses (Table 5). There were no significant differences in food intake among the groups ($F(3,42) = 2.67$, $p < .06$). The apparent reduction in intake observed in animals pretreated with the 1 ug naloxonazine dose (71% of control) failed to achieve statistical significance.

EXPERIMENT 2E: Central naloxonazine or naloxone and deprivation-induced intake.

Rationale: Intracerebroventricular administration of naloxone decreases feeding following deprivation (Jones and Richter, 1981). In order to obtain this effect, naloxone has been administered at the end of the deprivation period. The present study compared the effects of central administration of either naloxonazine or naloxone upon deprivation-induced intake when administered at the beginning of the deprivation period, 24 h prior to food reinstatement.

METHOD

Twenty-four cannulated rats were matched into three groups on the basis of their 24 h food intake. The groups received icv injections of either 50 ug of naloxonazine, 50 ug of naloxone, or vehicle respectively and then were deprived of food for 24 h. Following deprivation, food intake was assessed at 2 and 24 h.

RESULTS

Insert Table 6 here

TABLE 6. Reduction in 24 h deprivation-induced intake following central naloxonazine at 24 h but not 2 h following reintroduction of food.

	Post-deprivation (h) Intake (grams: SEM)	
Group (n)	2	24
Vehicle (9)	4.3 (0.4)	31.5 (1.2)
Naloxone (9)	4.7 (0.5)	28.2 (0.8)
Naloxonazine (8)	3.0 (0.7)	22.8 (3.9)+

Note 1. All injections were administered at the beginning of the deprivation period. + indicates a significant difference from vehicle (Dunn comparisons, $p < .01$).

Table 6 shows that there was a significant reduction in food intake of naloxonazine-treated animals at 24 h, but not at 2 h ($F(2,23) = 2.62$) following the deprivation period. Significant differences in food intake at 24 h occurred for the interaction between groups and conditions ($F(2,23 = 5.16$, $p < .01$), but not among groups ($F(2,23) = 1.75$) or treatment conditions ($F(1,23) = 2.43$). Pretreatment with naloxone at the beginning of the deprivation period, 24 h prior to the reintroduction of food, failed to alter deprivation-induced intake at either 2 or 24 h following reintroduction of food.

DISCUSSION

In assessing the effects of naloxonazine upon ingestive behaviors, it was found that intravenous administration a) was more effective than central administration in attenuating free feeding; b) was more effective than central administration in attenuating short-term hyperphagia induced by 24 h of food deprivation; but c) failed to alter the hyperphagic response following 2-DG administration. While naloxonazine exerted its hyperphagic effects over a 24 h period, the duration of naloxone-induced hypophagia was shown to be short-term.

The initial observation that systemic morphine increased food consumption in rats (Martin et al., 1963) together with the subsequent identification of opiate receptors and endogenous opioid ligands, provided a rationale for the understanding of a role for opioids in ingestive behaviors. It is now apparent that endogenous opioids play a modulatory role in free feeding as well as the hyperphagic responses which follow certain stressful situations. Systemic administration of the mu-receptor agonist morphine (Jaloweic et al., 1981; Sanger and McCarthy, 1980), kappa-receptor agonists ketocyclazocine, ethylketocyclazocine and cyclazocine (Lowy and Yim, 1982; Morley et al., 1982; Sanger and McCarthy, 1981), and the sigma-receptor agonist SKF-0047 (Gosnell et al., 1983) stimulate feeding in the rat, an effect which is reversed by naloxone. Intracerebroventricular administration of either beta-endorphin (McKay et al., 1981) or dynorphin (Morley and Levine, 1981) also induces naloxone-reversible increased feeding in the rat. Naloxone's short duration fo action

induces brief (1-2 h) decreases in feeding which are often followed by compensatory feeding (Cooper, 1980). Therefore, naloxone does not produce any long-lasting anorectic effects upon ingestion. In addition, the specificity of naloxone in reducing food intake has been questioned since several studies have reported nausea and learned taste aversions following naloxone administration (Frenk and Rogers, 1979; Goldberg, Morse, and Goldberg, 1976; LeBlanc and Cappell, 1975).

The results of Experiment 2A are in agreement with previous findings that opiate antagonists decrease free feeding. Free feeding was significantly reduced over a 24 h period following intravenous administration of naloxonazine (10 mg/kg), an effect presumably due to its long-term ability to block MU1 binding sites (Hahn and Pasternak, 1982). Vehicle-treated rats ate less than their baseline value over the 24 h period, but this inhibition was small and only observed in this particular group. It should be noted that the magnitude of inhibition induced by naloxonazine over a 24 h period is comparable to the magnitude of inhibition induced by naloxone over a short period. This suggests that both naloxone and naloxonazine are acting on similar mechanisms, presumably the MU1 sites, even though naloxonazine's effects are of longer duration. Intracerebroventricular naloxonazine at doses employed in the present study (Experiment 2D) failed to significantly reduce free feeding. A clear dose-response function was not apparent since the most effective central dose was 1 ug. This lack of congruence between intravenous and central routes of naloxonazine administration upon free feeding is in contrast to the situation with morphine analgesia, where both

routes of naloxonazine administration are equally effective. One possible explanation for this is that the icv route of administration does not allow access of naloxonazine to the appropriate anatomical loci mediating this feeding response. The intravenous route of administration, however, allows irrigation of many additional brain structures. Autoradiographical studies are needed to determine the distribution of naloxonazine following both routes of administration. Furthermore, definitive statements cannot be made regarding the lack of a central antagonist effect until larger subject samples over wider dose ranges and sampling periods are assessed.

Systemic administration of naloxone has been shown to reduce the hyperphagia associated with several environmental manipulations, including food deprivation (Holtzman, 1974; Brown and Holtzman, 1979; Frenk and Rogers, 1979; Lowy et al., 1980), 2-DG administration (Lowy et al, 1980; Sewell and Jawaharlal, 1980) and tail-pinch (Morley and Levine, 1980). Pretreatment with intravenous naloxonazine (10 or 20 mg/kg) but not naloxone (10 mg/kg) at the beginning of the deprivation period attenuated deprivation-induced hyperphagia 2 h after food reintroduction. Several lines of evidence suggest that these effects are mediated through naloxonazine's long-term ability to bind to MU1 sites. First, naloxonazine, but not naloxone, reduced deprivation-induced intake even if it was administered at the beginning of the deprivation period. This precluded the possibility that naloxonazine was exerting such effects by converting into naloxone. Second, since long-term pretreatment with naloxonazine and short-term pretreatment with naloxone produced similar magnitudes of

inhibition of deprivation-induced intake, it appears that they are acting on similar mechanisms, presumably MU1 opioid binding sites. Third, since long-term pretreatment with naloxonazine and short-term pretreatment with naloxone inhibited intake at 2 h but not 24 h following food reintroduction, this suggests that the MU1 site modulates short-term hunger signals, but is supplemented by other neural and/or hormonal mechanisms over the longer term.

Central administration of either naloxone (50 ug) or naloxonazine (50 ug) failed to alter deprivation-induced hyperphagia 2 h following food reinstatement to the same degree as intravenous injection. Previous studies have shown that the degree of inhibition of deprivation-induced intake is less following central as compared to systemic administration of naloxone (Jones and Richter, 1981). The small but significant reduction in naloxonazine-treated animals in deprivation-induced intake 24 h after food reintroduction is difficult to interpret, but may have to do with the clearance of the antagonist from the ventricular space. The site of action is as yet unknown and further dose-response curves and intracerebral injection studies should be carried out. Yet, it is important to note the comparison between consistent and inconsistent effects of central naloxonazine upon morphine analgesia and deprivation-induced intake respectively.

Naloxonazine (10 mg/kg) administered intravenously 24 h prior to 2-DG (400 mg/kg) failed to alter the hyperphagia observed 2 or 4 h following onset of glucoprivation. In fact, food intake was significantly increased at 4 h relative to vehicle-treated controls. In contrast, immediate pretreatment with naloxone significantly

decreased 2-DG hyperphagia at 2 and 4 h, confirming previous reports (Lowy et al., 1980; Sewell and Jawaharlal, 1980). Therefore, naloxonazine's effects upon ingestive behaviors dissociate between inhibition of free feeding or deprivation-induced hyperphagia and failure to alter 2-DG hyperphagia. On the other hand, naloxone decreases feeding in all three paradigms, indicating a role for opiate receptors in each. One explanation for the differences between the respective abilities of naloxone and naloxonazine to inhibit 2-DG-induced intake may involve opiate receptor sub-types with which these compounds interact. Since naloxonazine, like naloxone, decreases free feeding and deprivation-induced hyperphagia, this would suggest a role for MU1 binding sites in these two types of feeding. Since naloxone alone decreases the hyperphagic response following 2-DG, it would appear that naloxonazine-insensitive MU2 or other opioid binding sites mediate this effect. Future studies employing naloxonazine can determine whether the MU1 site mediates the feeding responses following administration of mu, kappa and sigma receptor agonists.

GENERAL DISCUSSION

The development of specific and long-lasting opiate antagonists has provided insight into the mechanisms through which opiates produce their effects. The present results have confirmed the importance of the MU1 opioid binding sites in the expression of opiate analgesia. Additionally, through the use of naloxonazine, they provide evidence of a role for these binding sites in ingestive processes. Although MU1 sites are involved in these two distinct behaviors, their mechanisms of action appear to be different. The opiate receptors involved in analgesia appear to be phasically activated, since naloxonazine attenuated morphine analgesia but failed to alter basal pain thresholds. In contrast, opiate receptors involved in feeding seem to be tonically active, since naloxonazine significantly reduced free feeding.

MU1 opioid binding sites have been implicated in opiate analgesia, free feeding and deprivation-induced hyperphagia. Future studies are planned to examine the role of these binding sites in several aspects of these behaviors. For example, the role of MU1 binding sites in so-called opiate and non-opiate forms of stress-induced analgesia (Lewis et al., 1980) needs to be evaluated. In addition, the effects of naloxonazine upon peptidergic analgesia should be evaluated to determine whether these analgesic responses possess an opioid component undetected by naloxone.

Many stressors induce hyperphagia and obesity which is reversed by naloxone. The present studies have demonstrated that MU1 sites are

involved in free feeding and deprivation-induced feeding, but not hyperphagia following 2-DG administration. This effect may be mediated through MU2 or other binding sites since the response is attenuated by naloxone. It is important to know under which conditions the MU1 sites mediate feeding and under which circumstances other receptor sites are involved. Perhaps certain characteristics of stress promote the influence of one type of binding site while other factors promote the involvement of another type of binding site. Through the use of naloxonazine, it should be possible to determine the role of the MU1 opioid binding sites in other behaviors as well, such as tolerance and dependence.

While long-lasting opiate antagonists such as naloxonazine provide information about the behavioral role of the MU1 opioid binding sites, the neuroanatomical localization of these binding sites is unknown. Autoradiographical studies employing labelled naloxonazine may provide us with this information. Although one can only speculate about their localization, possible loci of MU1 sites mediating opiate analgesia may be in areas of the brain stem such as the PAG and NRM since opiates injected into these areas produce naloxone-reversible analgesia. The MU1 sites involved in ingestive processes may be within the hypothalamus, specifically the PVN and VMH since opiates injected into these areas stimulate feeding. Once the precise localization of these binding sites is established, the localization significance may be derived from intracerebral microinjection studies. These anatomical studies are essential in understanding the opioid-mediated pathways influencing opioid-mediated behaviors

attributed to MU1 opioid binding sites.

In summary, the present studies have demonstrated the effectiveness of naloxonazine in modulating opiate analgesia and ingestive behaviors. Biochemical and pharmacological research in these areas is aimed at the development of powerful, yet non-addicting analgesics, as well as the development of safe anorectics in the control of obesity. However, the involvement of multiple receptor and transmitter interaction in these behaviors makes this a difficult task. In order for such medications to be developed, the basic receptor and transmitter mechanisms must be understood. Naloxonazine has proven to be a powerful tool in characterizing some of the opiate receptor mechanisms involved.

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