THE ROLE OF OPIOID RECEPTORS IN LORDOSIS BEHAVIOUR

By

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Abstract

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Opioid agonists such as heroin, morphine, methadone, Met\(^5\)-enkephalinamide, and \(\beta\)-endorphin have been shown to inhibit sexual behaviour in a variety of mammalian species. Although an extensive literature exists on the neuroendocrinology of opioid administration, little is known about the role of individual opioid receptors in the inhibition of sexual behaviour. This lack of information is due, in large part, to the lack of opioid receptor selectivity of the drugs used to study sexual behaviour.

Accordingly, the present experiments assessed the dose-response and time-course effects of several selective opioid peptides, administered intracerebroventricularly (icv), on the lordosis behaviour of ovariectomized rats rendered sexually receptive by estrogen and progesterone. In Experiments 1 through 4, lordosis behaviour was assessed in each female rat 15, 30, 60, 90, and 120 min after the infusion of different doses of a selective peptide. In Experiment 5, lordosis behaviour was measured 60 min after the infusion of effective doses of each of the peptides studied in the preceding experiments.

In Experiment 1, the effect of \(\mu/\delta\) receptor activation was assessed using \(\beta\)-endorphin. \(\beta\)-endorphin produced a dose-dependent dual effect on lordosis behaviour; a low dose (200 ng)
significantly inhibited lordosis, whereas a higher dose (2000 ng) produced a significant facilitation. Experiment 2 examined the effect of the selective μ receptor agonist, morphiceptin, on lordosis behaviour. Morphiceptin also produced a dose-dependent dual effect in which the lowest dose (20 ng) significantly inhibited, whereas the two higher doses (200, 2000 ng) significantly facilitated lordosis. In Experiment 3, the selective δ receptor agonist, δ-receptor peptide, significantly facilitated lordosis behaviour at all doses. In contrast, in Experiment 4 the selective κ receptor agonist, dynorphin 1-9, had no significant effect on lordosis behaviour. Finally, in Experiment 5, the ability of the opioid receptor antagonist naloxone to reverse the effects of β-endorphin, morphiceptin, and δ-receptor peptide observed in the first four experiments was determined. The effects of each peptide were replicated and were reversed by naloxone, indicating that the effects observed in the first four experiments were reliable and that they were produced by the activation of opioid receptors. Naloxone alone, however, had no effect on lordosis behaviour.

These results indicate that the selective activation of opioid receptors differentially affects lordosis behaviour in female rats. It appears that binding to high-affinity μ₁ receptors exerts an inhibitory influence on lordosis, whereas binding to low-affinity μ₂ receptors or δ receptors exerts a facilitatory influence. Binding to κ receptors does not appear to affect lordosis behaviour. The failure of naloxone alone to affect lordosis behaviour suggests that endogenous opioid systems do not exert a tonic inhibitory or facilitatory
influence in ovariectomized rats primed with estrogen and progesterone. The present experiments demonstrate the importance of using highly selective opioid receptor ligands in the study of the behavioural effects of opioid drugs.
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I dedicate this thesis to my parents.
Foreshadow

Dr. Strauss: It seems to be strange that the alien is interested in heroin. But there could be a lot of reasons for that. We know now, for example, because of the research of a few American scientists in the late 1970s, that there are special receptors, opiate receptors, in the human brain....

Ms. Goldman: "Well, what are all these opiate receptors doing sitting around in the human brain? Waiting for someone to come along and... give them heroin?"

Dr. Strauss: "Some physicians think that there is a naturally-occurring molecule in the human body with nearly the same molecular structure as opiates."

Ms. Goldman: "You mean to say that opium occurs naturally in the human body?"

Dr. Strauss: "Not opium; I said nearly the same molecule with nearly the same properties. Opium users have said that the drug creates a similar feeling to what people experience during orgasm. It could be that this molecule is released into the brain during orgasm."

Ms. Goldman: "During orgasm? Well... that's very interesting."

Introduction

For centuries opioids have provided a refuge from pain and stress; their use as analgesics, anxiolytics, and narcotics has been well-documented (Krueger, Eddy, & Sumwalt, 1941, 1943; Stimmel, 1975; Wickler, 1980). Opioids have also enjoyed use solely for pleasure or to enhance artistic creativity (Chessick, 1960; DeQuincey, 1821; Kolb, 1925; Larner, 1967; Rado, 1933). In the absence of physical pain, a common feature of acute opioid use has been a brief, but very intense euphoria followed by a prolonged period of relaxation, sedation, and sense of well-being (Mirin, Meyer, Mendelson, & Ellingboe, 1980). Throughout history, opioid users have described these effects in sexual terms, often equating them with sexual orgasm (Becker, 1903; DeQuincey, 1821; Jones, 1700; Jones & Jones, 1977; Kaan, 1891).

In contrast to descriptions of an orgasm-like euphoria with acute opioid administration, chronic opioid use has long been associated with a progressive and insidious deterioration of sexual functioning (Ashworth, 1914; Bloom & Butcher, 1970; Cushman, 1972; Coltman, 1890; DeLeon & Wexler, 1973; Garbutt & Goldstein, 1972; Guerra, 1974; Happel, 1892; Jones, 1700; Jones & Jones, 1977; Larner, 1967; Mathis, 1970; Noirot, 1902; Pace, 1892; Passower, 1893; Wholey, 1912; Wielund & Yunger, 1970). The long-term use of opioids by male addicts has been associated with the elimination of sexual dreams, delayed ejaculation, decreased ejaculate volume, anorgasmia, inhibited sexual desire, and in some cases infertility. Similarly, the long-term use of opioids by female addicts has been associated with the elimination of sexual dreams, amenorrhea, anorgasmia, inhibited
sexual desire, and in some cases infertility. Eventually, female
and male addicts experience a severely diminished capacity for
sexual arousal; for them, the use of opioids seems to "replace
sex" (Jones & Jones, 1977; Rado, 1933). Subsequent withdrawal
from opioids, either through drug abstinence or challenge with
opioid antagonists, is characterized by a gradual restoration of
sexual interest and functioning.

Although the central and peripheral mechanisms underlying
the classic analgesic effect of opioids have been described in
detail (Akil & Watson, 1979; Basbaum & Fields, 1984; Watkins &
Meyer, 1982; Wickler, 1980), it is not yet known how opioids
produce an orgasm-like euphoria in the absence of pain, or
whether the analgesic or euphoric actions of opioids are related
to the inhibition of sexual behaviour. In fact, it is only
within the last decade that the effect of opioids on sexual
behaviour has received extensive experimental attention.
However, such research has not kept pace with the ever-expanding
knowledge of opioid pharmacology and biochemistry (cf. Pfaus &
Gorzalka, 1986). The experiments reported in this thesis are a
first attempt at bridging this gap in information by addressing
the fundamental question of whether opioid receptors are
differentially involved in the inhibition of sexual behaviour.
This question is addressed in the present experiments by testing
the effect of several relatively selective opioid receptor
ligands on lordosis behaviour in female rats. In order to
provide a broad background for the rationale and discussion of
the present series of experiments, it is necessary to briefly
review the pharmacology and biochemistry of opioids.
The Pharmacology and Biochemistry of Opioids

History. Opium, from the Greek opop meaning "juice," is the dried, powdered form of the milky exudate obtained from the seed capsules of the poppy plant. It contains over 20 alkaloids, some of which are powerfully psychoactive. Before the isolation and production of these particular alkaloids, opium and its extracts, eg., tincture of opium or laudanum, were used widely as analgesics, sedatives, narcotics, and aphrodisiacs.

In 1805, Sertturner in Germany produced morphine, named in honour of Morpheus, the god of dreams in Ovid's play Metamorphoses, from the alkaline base obtained after separating meconic acid from opium. His procedure, which generally involved the separation of acid constituents from opium, was used by Robiquet in France, who isolated narcotine in 1817 and subsequently codeine in 1832 (Krueger et al., 1941).

Heroin (diacetylmorphine) was first synthesized in 1874 by Wright in England, who boiled morphine in acetic acid to acetylate the hydroxyl groups in the molecule and facilitate lipid permeability. It was not until 1897, however, that heroin was marketed to the public by the Beyer Pharmaceutical Company in Elbersfeld, Germany. The effectiveness of heroin as an analgesic was so great that in 1906 the American Medical Association officially approved its general use and recommended it over morphine for patients suffering from chronic pain. Although heroin was officially banished from medical use in the United States during the 1920s and in Canada in the 1940s, specifically because of its powerful abuse potential, it has remained the opioid of choice for recreational use.
Great advances were made in understanding the biological disposition and the structure/activity relationships of various opioid alkaloids over the past 40 years. These advances provided the critical information needed to synthesize analgesics with diminished abuse potential. Some of the analgesic compounds synthesized from morphine or thebaine during the 1950s and 1960s included the benzomorphans, eg., pentazocine; the phenylpiperidines, eg., meperidine; the diphenylamines, eg., methadone; the morphans, eg., levorphanol; and the 6,14-endo-ethynyloripavines, eg., etorphine. In rats, many of these compounds could be discriminated from morphine and heroin at very low doses, yet were as effective as morphine in attenuating withdrawal symptoms in morphine-dependent animals (Way, 1979). More importantly, many of these compounds could not serve as reinforcers in self-administration paradigms, indicating both a diminished abuse potential and a possible dissociation between analgesia and euphoria.

The early 1960s saw the clinical application of this work. Methadone and some of the more active benzomorphans, eg., cyclazocine, were introduced as drug maintenance therapy for heroin addiction. The relative analgesic properties of synthetic opioids were assessed in humans. In the late 1960s, the first "pure" opioid antagonist, N-allylnoroxymorphone (naloxone) was introduced. The biochemical basis of opioid tolerance and dependence was examined, as was the mediating role of other putative neurotransmitter systems, eg., dopamine or serotonin. Most importantly, central and peripheral sites of the activity of opioids were identified in the late 1960s and early 1970s,
which led several investigators to search for "endogenous opioids" and drug-specific receptor proteins.

**Chemistry.** The term **opiate** refers specifically to the alkaloids obtained directly from the juice of the poppy. The generic term **opioid** refers to any compound with opiate-like activity that is reversed by a general opioid antagonist such as naloxone (Goldstein, 1975). Opioids may also possess nonopioid actions which, by definition, cannot be reversed with a general antagonist.

Opioid peptides are specific amino acid sequences that possess opiate-like pharmacological activity which is reversed with naloxone. All endogenous opioid peptides are cleaved by proteolytic enzymes, eg., aminopeptidase or enkephalinase, from larger precursory hormones (see DeWied & Jolles, 1982; Marx, 1983; O'Donohue & Dorsa, 1982, for reviews). Briefly, β-endorphin and methionine-enkephalin (Met^5^-enkephalin) are derived from Proopiomelanocortin, a 30 kD protein from which is also cleaved the ACTH and MSH peptide sequences. Met^5^-enkephalin is also derived from Proenkephalin, a 28 kD protein from which leucine-enkephalin (Leu^5^-enkephalin) is derived. Finally, the dynorphin sequences, eg., dynorphin 1-17, dynorphin 1-9, leumorphin, are cleaved from Prodynorphin, a 29 kD protein. Endogenous opioid peptides are generally produced in the anterior lobe and pars intermedia of the pituitary and are released into the portal vasculature. A relatively small but important proportion of the total endogenous opioid peptide production occurs within perikarya and on cell membranes in several other brain areas (see below).
Opioid Receptors. Endogenous opioid receptors were discovered concurrently in the myenteric plexus of the guinea-pig ileum (Kosterlitz, Lord, & Watt, 1973) and in the brain tissue of monkeys (Pert & Snyder, 1973). Subsequently, several brain-borne peptides were isolated and classified as possible endogenous ligands for these receptors. Among the early candidates were Met⁵-enkephalin and Leu⁵-enkephalin, two pentapeptides with potent, but short acting opioid activity (Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975). β-endorphin, a 31-amino acid peptide representing positions 61-91 of the β-lipotropin molecule, was found to possess opiate-like activity very similar to that of morphine (Hughes, 1975; Pasternak, Goodman, & Snyder, 1977; Teschmacher, Opheim, Cox, & Goldstein, 1975). Several studies followed that localized opioid receptors in various brain areas using autoradiographic techniques (Pert, Kuhar, & Snyder, 1976; Simantov, Childers, & Snyder, 1978). It soon became apparent, however, that more than one opioid receptor existed.

Kosterlitz and associates discovered that some endogenous peptides bound to peripheral tissue preparations differently. The enkephalins, for example, were found to bind with higher selectivity, i.e., at lower concentrations, to receptors in the mouse vas deferens than to receptors in either the rat vas deferens (selective for β-endorphin) or the guinea-pig ileum (selective for morphine). Other opioids, such as the benzomorphan drugs ketazocine and ketocyclazocine, were found to bind with high affinity to receptors in the rabbit vas deferens but not at all to receptors in the mouse vas deferens.
In 1976, Martin and associates proposed that three functional classes of opioid receptor existed and named them after prototypic ligands: µ receptors (after morphine); κ receptors (after ketazocine); and σ receptors (after SKF-10047 or N-allylnormetazocine) (Martin, Eades, Thompson, Huppler, & Gilbert, 1976; Gilbert & Martin, 1976). Kosterlitz added a fourth class, the δ receptor (after mouse vas deferens), to explain the preferential binding of the enkephalins (Paterson et al., 1983). The dynorphin peptide sequences were discovered in the late 1970s and were subsequently shown to be endogenous ligands for κ receptors (Corbett, Paterson, McNight, Magnan, & Kosterlitz, 1982; Oko, Negishi, Suda, Sawa, Fujino, & Wakimasu, 1982; Paterson, Robson, & Kosterlitz, 1983). In the early 1980s, it was also proposed that β-endorphin bound to a macromolecular receptor complex, referred to as the ε receptor (after endorphin), consisting of a protein-bound δ and a lipid-bound µ receptor (Smith, Lee, & Loh, 1983; Zuckin & Zuckin, 1984).

Generally, the discovery and classification of opioid receptor subtypes has been hastened by the development of agonists and antagonists have relatively selective affinities. The receptors described above were initially characterized using both in vitro binding assays and in vivo bioassay techniques, eg., measures of analgesia such as the tail-flick test. The presence of heterogeneous populations of opioid receptors was later confirmed in various brain areas using in vitro selective protection techniques, in which the binding of a drug to a particular receptor is examined following the saturation of
other receptor types in situ with other relatively selective drugs. More recent evidence, gathered with the use of more selective opioid receptor ligands, has suggested further subdivisions of opioid receptors. There are currently believed to be two subclasses of $\mu$ receptor, a high-affinity ($\mu_1$) site and a low-affinity ($\mu_2$) site. The $\mu_2$ site corresponds to the morphine-selective receptor originally described by Kosterlitz et al. (1973) in the guinea-pig ileum. The $\mu_1$ site, originally referred to as the "high-affinity opiate binding site" by Pasternak and Snyder (1975), was found to be selectively blocked in several studies by the long-acting antagonist naloxazone, a hydrazone derivative of naloxone (Hazum, Chang, Cuatrecasas, & Pasternak, 1981; Ling, Spiegel, Nishimiri, & Pasternak, 1982; Pasternak, Childers, & Snyder, 1980a,b; Wolozin & Pasternak, 1981). Naloxazone was also shown to block the analgesia produced by low doses of morphine without affecting the respiratory depression produced by higher doses (Pasternak et al., 1980a).

Naloxazone also differentially displaces radiolabelled D-Ala$^2$-Met$^5$-enkephalinamide and dihydromorphine binding in various regions of rat brain (Zhang & Pasternak, 1980). Brain areas rich in $\mu_1$ receptors, eg., the hypothalamus and spinal cord, were most sensitive to the effect of naloxazone, whereas areas containing both $\mu_1$ and $\mu_2$ receptors, eg., the striatum and thalamus, were less affected. Brain areas rich in $\delta$ receptors, eg., the cortex and midbrain, were markedly insensitive to naloxazone. Although the functional role of $\mu$ receptor subtypes has not yet been established, it has been suggested that $\mu_1$ receptors may mediate classic opioid effects such as analgesia,
whereas $\mu_2$ receptors may mediate effects similar to those produced by $\delta$ receptor activation (Chaillet, Couland, Zajac, Fournie-Zaluski, Constantin, & Roques, 1984; Pasternak, Gintzler, Houghten, Ling, Goodman, et al., 1983; Pazos & Florez, 1984). In fact, recent biochemical evidence indicates that the $\mu_2$ receptor is structurally similar to the $\delta$ receptor (Rothman, Danks, Herkenham, Jacobson, Burke, & Rice, 1985).

Although the $\sigma$ receptor was originally classified by Gilbert and Martin (1976) as an opioid receptor, the drugs that selectively bind to it, eg., SKF-10047 or phencyclidine, do not possess the pharmacological profile of other opioids. Instead of analgesia, these drugs produce hallucinations and sedation, both of which may involve an action on other neurotransmitter systems, eg., serotonin. Furthermore, it has recently been shown using selective protection techniques in vivo that the sedative effect of SKF-10047 is mediated through partial agonist activity at $\kappa$ receptors (Khazan, Young, El-Fakany, Hong, & Calliagro, 1984). The status of the $\sigma$ receptor as a "true" opioid receptor, therefore, remains in question.

The central distribution of opioid receptors has also been of great interest. Opioid receptor subtypes have been found to occur together on the same membrane in some brain regions and separately in others. In mammals, opioid receptors have been localized in the olfactory bulbs; the projection areas of the vomeronasal organ, a chemosensory system implicated in the processing of pheromonal stimuli; the cortex; ventral septal nuclei; nucleus accumbens; periventricular nucleus of the thalamus; striatum; preoptic area; mediobasal hypothalamus;
median eminence; pituitary; amygdala; mesencephalic central grey area (MCG); ventral tegmentum; locus coeruleus; reticular formation, e.g., the dorsal raphe and the nucleus reticularis gigantocellularis; cerebellum; the spinal nucleus of the trigeminal nerve; and diffusely throughout spinal dorsal horn nuclei (see Akil & Watson, 1979; Bloom, 1983; Goodman & Snyder, 1982; Khachaturian, Lewis, Schafer, & Watson, 1985; Robson, Foote, Maurer, & Kosterlitz, 1984; Szara, 1982, for reviews). In the cortex, κ receptors are found largely in laminae I, V, and VI, whereas a predominance of δ and some μ receptors are found in laminae II, III, IV, and V (Foote & Maurer, 1982; Goodman, Snyder, Kuhar, & Young, 1980). Opioid receptors that have been implicated in the regulation of pituitary function are located in both the arcuate nucleus of the hypothalamus and in the median eminence and appear to be of the μ and δ type or the epsilon complex because β-endorphin, Met⁵-enkephalin, and morphine all bind to these sites. κ receptors have not been found in the mediobasal hypothalamus.

Peripherally, opioid receptors have been found in the myenteric plexus of the ileum, the vas deferens, the heart, kidneys, and gut (Jaffe & Martin, 1980). However, the opioid receptor subtypes found in these areas, especially in the ileum and vas deferens, differ among species (Paterson et al., 1983). Such differences have made the functional role of peripheral opioid receptors difficult to assess.

The diffuse central distribution of opioid receptors has also made it difficult to identify specific central mechanisms through which opioids influence behaviour. Nevertheless, as
specific receptor pathways become apparent, the ability to "map" opioid effects with selective ligands increases.

**Central and Peripheral Effects.** Although opioids are best known for their analgesic, narcotic, and euphoric actions, they produce several other notable effects in the central and peripheral nervous systems. Pupillary constriction is a prominent effect in humans and forms a diagnostic criterion of opioid overdose in conjunction with respiratory depression. Respiratory depression produced by opioids is a result of the inhibition of medullary sites responsible for the breathing reflex. In subanalgesic doses, morphine and other opiates act as cough suppressants (Jaffe & Martin, 1980).

Opioids decrease glandular secretions and contract smooth muscle fibres. Both of these effects, along with decreased intestinal peristalsis, result in constipation, a common side effect of opioid administration. Opioids also produce vestibular stimulation, which results in nausea and vomiting in some users. Hypotension and decreased blood pressure are other prominent effects along with hypothermia (Jaffe & Martin, 1980).

Opioids also disrupt neuroendocrine function. For example, heroin and methadone both decrease serum luteinizing hormone (LH) and testosterone levels, although the inhibitory effect of methadone on testosterone secretion occurs at high doses only (Azizi, Vaginakis, Longcope, Ingbar, & Braverman, 1973; Cicero, Schmoeker, Meyer, Miller, Bell, et al., 1986; Mendelson, Ellingboe, Kuenhle, & Mello, 1980).

Endogenous opioid systems have been implicated as putative neurotransmitter or neuromodulatory hormonal substrates of a
wide variety of psychologically relevant phenomena: Pain mediation, tolerance and dependence to exogenously administered opioid drugs, regulation of neuroendocrine function and dysfunction, sedation, sleep, euphoria, reinforcement mechanisms, consummatory behaviour, sexual orgasm, sexual dysfunction, anxiety, learning and memory, and assorted psychopathological states (see Cooper & Martin, 1982; Olson, Olson, & Kastin, 1984; Pfaus & Gorzalka, 1986; Reid & Siviy, 1983; Rossier & Bloom, 1979; Szara, 1982, for reviews).

Opioids and Sexual Behaviour

The impetus to study the effect of opioids on sexual behaviour comes from a long history of anecdotes and clinical accounts (Pfaus & Gorzalka, 1986). As mentioned previously, acute administration of heroin is reported by users to produce an immediate, orgasm-like "rush" of euphoria that gradually tapers off into feelings of general relaxation and contentment. Although long-term opioid use has not been reported to diminish this subjective experience, provided an effective dose is maintained, the actual sexual motivation and functioning of the user diminishes. A large part of the psychiatric maladjustment of heroin addicts is based on their inability to maintain intimate and supportive relationships. The progressive decline, first in the capacity to enjoy, and then to maintain, sexual activity is an important factor. Furthermore, the sexual gratification experienced by many addicts following intravenous heroin administration appears to play a major role in the maintenance of opioid use (Chessick, 1960; Rado, 1933).
As the debilitating effects of long-term opioid use in humans were first examined clinically during the early part of this century, comparative animal models of those effects were sought in order to examine more closely the mechanisms of action of opioid drugs (Kraus, 1918). The acute and chronic effects of opiates were originally studied in dogs, mice, rats, rabbits, monkeys, and chimpanzees, and were shown to mimic many of the symptoms observed in humans. Likewise, many of the symptoms of opiate withdrawal, e.g., restlessness, diarrhea, respiratory alterations, muscular twitching, and rigidity, were evident in all of the species examined.

Sexual behaviour was usually given only a passing mention in those studies. Single injections of low doses of morphine or heroin (usually 2 to 5 mg/kg) were generally reported to have no effect upon sexual behaviour. Long-term administration of higher doses of morphine or heroin (up to 70 mg/kg) invariably resulted in an inhibition of sexual activity in female and male dogs (Eddy & Reid, 1939; Plant & Pierce, 1928), female mice (Ko, 1935), female and male monkeys (Seevers, 1936; Tatum, Seevers, & Collins, 1929), chimpanzees (Spragg, 1940), and also the elimination of sexual odourants in the vaginal secretions of intact dogs (Hashimoto, 1938). During subsequent drug abstinence, a restoration of sexual activity was observed in all species. Interestingly, the restored interest in copulation and masturbation observed in male monkeys during drug abstinence was reportedly preceded by a period of spontaneous ejaculations not dependent upon genital stimulation (Tatum et al., 1929). This effect was identical to that later reported in human males.
during heroin withdrawal (Greenberg, 1985).

Reports of an inhibitory effect of long-term opioid administration on female sexual behaviour, however, was controversial in the early literature. Although overt solicitation was not generally observed in female dogs or rats during long-term morphine treatment, a number of these animals reportedly became pregnant and bore healthy litters during treatment (Myers & Flynn, 1928; Plant & Pierce, 1928). Myers (1931) tested the effect of long-term morphine administration (50 to 70 mg/kg/day) in intact female albino rats for a period of 5 months, beginning 6 days after weaning. No evidence was found supporting the predictions of retarded growth, inhibition of the estrous cycle, or an inability to mate and subsequently bear healthy litters. Although it could be argued that tolerance to the inhibitory effects of morphine on ovulation or sexual behaviour might have developed long before the fifth month of drug treatment, those data coincided with reports in the human clinical literature that pregnancy did indeed occur in some female heroin addicts, who presumably adjusted their doses to avoid tolerance effects (McGuigan, 1929; Wholey, 1912).

Unfortunately, the effects of opioids on sexual behaviour in laboratory animals did not receive further attention until the early 1970s, when Cushman, and later Mendelson and associates, published detailed analyses of endocrine dysfunction in human heroin addicts (Cushman, 1972; Mendelson, Mendelson, & Patch, 1975; Mendelson, Meyer, Ellingboe, Mirin, & McDougle, 1975). Those reports were followed by studies demonstrating a similar effect of opioids in rats, eg., decreases in serum LH or
testosterone levels (Cicero, Wilcox, Bell, & Meyer, 1976; Tokunaga, Muraki, & Hosoya, 1977). Because of these similarities, rats, and subsequently hamsters, became the animal models of choice in the modern literature, although nonhuman primates have been used occasionally. In the late 1970s and early 1980s, parametric studies of dose, time course, route of administration, in addition to the acute and long-term inhibitory effect of morphine on copulatory behaviour in male rats began to appear in the literature (Hetta, 1977; Kumar, Mumford, & Teixeira, 1977; McIntosh, Vallano, & Barfield, 1980; Meyerson, 1981; Mumford & Kumar, 1979). Inhibitory effects of morphine were also reported on the lateral displacement of the tail during copulation in female golden hamsters (Ostrowski, Stapleton, Noble, & Reid, 1979), and on lordosis, the concave arching of the back and lifting of the anogenital region to facilitate penile intromission, in female rats (Pfaus & Gorzalka, 1986; Wiesenfeld-Hallin & Sodersten, 1984). Inhibitory effects of methadone were also reported on autosexual behaviour in male macaques (Crowley, Stynes, Hydinger, & Kaufman, 1974) and on copulatory behaviour in male golden hamsters (Murphy, 1981).

Although opioid peptides such as β-endorphin or D-Ala²-Met⁵-enkephalinamide have been reported to inhibit copulatory behaviour consistently in male rats (McIntosh, et al., 1981; Meyerson, 1981; Meyerson & Terenius, 1977; Pellegrini-Quarantotti, Corda, Paglietti, Biggio, & Gessa, 1979), the effects of opioid peptides on lordosis behaviour in female rats are more difficult to interpret.
Low doses of β-endorphin have been reported to inhibit the display of lordosis in ovariectomized (OVX), steroid-primed rats when infused into the MCG (Sirinathsinghji, 1984; Sirinathsinghji, Whittington, Audsley, & Fraser, 1983). Sirinathsinghji and associates demonstrated this effect in females that received both estrogen and progesterone or demonstrated this effect in females that received both estrogen and progesterone or estrogen alone. When β-endorphin (250 ng) was infused into the MCG, mean lordosis quotients, that is, the mean lordosis/mount ratios, were significantly reduced in both groups of rats within 30 min. In females that received estrogen treatment alone, lordosis behaviour was abolished within 2 hr and remained so for 7 hr. In females that received both estrogen and progesterone treatment, lordosis behaviour was significantly, but not completely inhibited for 4 hr. During this time, females significantly increased the frequency of active rejections of the stimulus male, eg., displayed boxing stances, crouching, etc., when compared with control females that received saline infusions. The inhibitory effect of β-endorphin in both steroid treatment groups was reversed with naloxone (2.5 mg/kg, ip), administered 15 min before β-endorphin.

Wiesner and Moss (1984) described a similar inhibition of lordosis behaviour in OVX rats primed with estrogen and progesterone when a very low dose of β-endorphin (100 ng) was infused into the third ventricle. Lordosis quotients were significantly reduced in contrast to those of saline treated control rats 15 and 45 min after infusion. At 75 and 135 min,
However, lordosis quotients had risen to control levels. The inhibition of lordosis at 15 and 45 min was reversed by naloxone (2 mg/kg, sc) administered concurrently with β-endorphin.

The difference in the duration of inhibition between these data and those reported by Sirinathsinghji (1984) and Sirinathsinghji et al. (1983) could reflect differences in dose, however, a more likely explanation is that β-endorphin was administered to different parts of the brain. It is well-known, for example, that the cerebral ventricles contain a high concentration of various proteolytic enzymes. Although hypothalamic structures adjacent to the third ventricle in rats also contain opioid receptors (Bugnan, Bloch, Lenys, Goudet, & Fellman, 1979; Grandison, Fratta, & Guidotti, 1980), peptides infused into the ventricles might be degraded more rapidly than those infused into discrete brain areas such as the MCG. Hence it might be expected that the behavioural effects of peptides infused into the ventricles would be of comparatively short duration.

It should not be construed from the explanation presented above that dose parameters are unimportant. In fact, they may be critical in determining whether β-endorphin inhibits or facilitates lordosis in female rats. Preliminary experiments conducted for this thesis demonstrated that 2 μg of β-endorphin facilitates lordosis 60 min after infusion into the lateral ventricles of OVX rats primed with estrogen and progesterone. This confirmed earlier unpublished observations from our laboratory that 2 μg of β-endorphin facilitates lordosis in OVX rats primed with estrogen alone. These results raise several
interesting questions. Because β-endorphin interacts with both μ and δ receptors it could be that the dual effect of β-endorphin on lordosis behaviour reflects a differential activation of these receptors. Another possibility is that β-endorphin facilitates lordosis by activating an anatomically distinct population of opioid receptors near the lateral ventricles. Answers to either of these questions would have important implications, not only for the understanding of the opioid receptor mechanisms involved in the modulation of lordosis, but for a more general understanding of opioid receptor interactions. Although no definitive answers exist, several hints have been presented.

In addition to β-endorphin, Sirinathsinghji (1984) tested the effect of Met5-enkephalin (0.5 or 10 μg) or dynorphin 1-17 (0.25 or 10 μg) on lordosis in OVX rats primed with estrogen alone. Although Met5-enkephalin binds to both μ and δ receptors, it has an affinity 10 times greater for δ than μ receptors (Paterson et al., 1983) and its duration of action at both receptors is relatively short (Hughes, 1983). Dynorphin 1-17 is rapidly cleaved into dynorphin 1-9 or dynorphin 1-8, both of which possess very potent, but relatively short action on κ receptors (Griffiths & McDermott, 1984; Paterson et al., 1983). Sirinathsinghji found no effect of either peptide on lordosis 2 hr after infusion into the MCG. Thus Sirinathsinghji concluded that only μ receptor activation in the MCG may be necessary for the inhibition of lordosis in female rats by β-endorphin.

Although this is a tempting hypothesis, a potential flaw existed in the design of those experiments that casts some doubt
on the notion that δ or κ receptors have no role in the modulation of lordosis behaviour. Because Met⁵-enkephalin and dynorphin 1-17 are relatively short-acting peptides, it is not clear whether relatively selective δ or κ receptor activation actually had no effect on lordosis or whether both peptides had been cleaved into inactive metabolites long before lordosis was tested. Time-response analyses of the effects of short-acting peptides may require shorter observation intervals. Indeed, another relatively selective κ ligand, leumorphin, has been reported to produce a short-term facilitation of lordosis in OVX, estrogen-primed rats (Imura, 1985; Suda, Nakao, Sakamoto, Morii, Sugawara, & Imura, 1986). In addition, peripheral administrations of estrogen are known to increase endogenous Met⁵-enkephalin levels in the MCG (DuPont, Borden, Cusan, Merand, LaBrie, & Vaudry, 1980) and to increase the synthesis of Proenkephalin in the ventromedial hypothalamus (D. Pfaff, 1986, personal communication). Both of these areas have been implicated in the central hormonal regulation of lordosis behaviour in female rats (Pfaff, 1980). Thus, it may be that selective δ or κ receptor activation serves to potentiate, rather than diminish, the display of lordosis. Administration of Met⁵-enkephalin to the MCG, however, had no effect on lordosis in OVX rats primed with estrogen and progesterone in a single test 4 hr after the concurrent administration of Met⁵-enkephalin and progesterone (Sirinathsinghji, 1984). It is not clear why Sirinathsinghji chose to administer Met⁵-enkephalin at the same time as progesterone priming and not 3 to 4 hr after progesterone, when female rats normally begin to display
consistent patterns of lordosis behaviour. Once again, the possibility that Met⁵-enkephalin had been cleaved into an inactive metabolite during the 4-hr period between infusion and lordosis testing cannot be discounted.

To summarize, a major focus of research concerning the effect of opioids on sexual behaviour has been the categorization of the effect of various opioid agonists on patterns of sexual behaviour in laboratory animals. The promise of such research has been to identify the physiological or pharmacological mechanisms through which opioids inhibit sexual behaviour. Although distinct pharmacological classes of opioid drugs have been shown to inhibit sexual behaviour in a variety of mammalian species, our knowledge of the mechanisms through which opioids exert such an inhibitory action remains poor. Differences in experimental procedure, most notably in dose, time course, route of administration, sex of species, and dependent measure, further confuse matters. However, such differences have provided the necessary inconsistencies to spark new questions and new research designs.

Rationale

Our inability to treat the sexual dysfunctions commonly associated with long-term opioid use stems in part from a general lack of knowledge concerning the central or peripheral mechanisms that underly the inhibition of sexual behaviour produced by opioid drugs. For example, it is currently impossible to determine the structure/activity relationship of opioid agonists to sexual behaviour. Although agonists such as
heroin, morphine, methadone, Met\textsuperscript{5}-enkephalinamide, and \( \beta \)-endorphin inhibit sexual behaviour, they differ widely in chemical structure. Similarly, it is difficult to relate the inhibition of sexual behaviour produced by opioid agonists to the activation of specific opioid receptors. This difficulty is due, in large part, to a lack of receptor selectivity in the opioids listed above. Morphine, for example, is 50 times more potent at \( \mu \) receptors than at \( \delta \) receptors, whereas \( \beta \)-endorphin has an approximately equal affinity for \( \mu \) and \( \delta \) receptors (Paterson et al., 1983; Smith et al., 1983). Clearly, a systematic analysis of the opioid receptors that may be involved in the regulation of sexual behaviour is not possible using these drugs alone. However, the isolation and classification of peptides that act as endogenous ligands for opioid receptors has hastened the development of synthetic peptide sequences that possess a relatively high affinity for different opioid receptors. A systematic analysis of the role of opioid receptors in the inhibition of sexual behaviour using highly selective receptor ligands could provide important clues for the structure/activity relationship of opioid receptor ligands to sexual behaviour, in addition to a more comprehensive understanding of the regional distribution of opioid receptors involved in the inhibition of sexual behaviour.

The lordosis posture assumed by sexually receptive female rats which facilitates penile intromission by sexually active males is ideally suited for an analysis of the effects of selective opioid receptor ligands. Lordosis quotients can be determined in a manner of minutes, depending upon the sexual
vigor of the stimulus male. This makes lordosis quotients an excellent dependent measure in time course analyses, especially of peptides that may possess relatively short action. In addition, the neuroanatomic pathways involved in the central and peripheral expression of lordosis behaviour have been studied in detail (Pfaff, 1980). Many of the central sites involved in the hormonal regulation of lordosis behaviour, especially the MCG and ventromedial hypothalamus, in addition to brainstem areas such as the lateral vestibular nucleus and the nucleus gigantocellularis, contain immunoreactive opioid peptide-containing neurons and are rich in opioid receptors (Atweh & Kuhar, 1977; Bugnan et al., 1979; Finley, Lindstrom, & Petrusz, 1981; Finley, Maderdrut, & Petrusz, 1981; Hollt, Haarman, Boverman, Terlicz, & Herz, 1980; Khachaturian et al., 1985). This suggests that endogenous opioid peptides may be naturally involved in the central regulation of lordosis behaviour. However, as mentioned earlier, although the effects of opioid peptides such as β-endorphin, Met⁵-enkephalin, dynorphin 1-17, and leumorphin have been studied on lordosis behaviour, those effects remain controversial. A systematic analysis of the involvement of opioid receptors in lordosis behaviour, using highly selective receptor ligands, would clearly help to resolve the controversy.

Accordingly, in the present series of experiments, opioid peptides that are highly selective ligands for µ receptors (morphiceptin), δ receptors (δ-receptor peptide), κ receptors (dynorphin 1-9), and the µ/δ receptor complex (β-endorphin) were administered to the lateral ventricles of OVX, steroid-primed
rats in an effort to determine which opioid receptors are involved in the central regulation of lordosis behaviour. In Experiments 1 through 4, dose-response and time course analyses were conducted on the effects of each peptide on lordosis behaviour. In Experiment 5, the ability of the opioid antagonist naloxone to reverse the effects of each peptide was tested. The results of these experiments demonstrated clearly that \( \mu, \delta \), and \( \kappa \) opioid receptors are differentially involved in the central regulation of lordosis behaviour.
General Methods

Animals and Surgery

The female Sprague-Dawley rats that served as subjects were obtained from Charles River Canada, Inc., Montreal. At approximately 100 days of age, all subjects were bilaterally OVX under sodium pentobarbital anesthesia (Somnital, 40 mg/kg, ip) and implanted with 23-gauge, stainless-steel guide cannulae aimed at the right lateral ventricles. Briefly, the surgical procedure for guide cannula implantation involved placing each female rat in a stereotaxic apparatus with the incisor bar set 5 mm above the interaural line. The scalp was cut, retracted, and 4 stainless-steel anchor screws were installed into the top of the skull. A burr hole was drilled into the skull through which the guide cannula was lowered into place. Each guide cannula was positioned according to the atlas of Pellegrino, Pellegrino, and Cushman (1979), with the tip of the cannula protruding approximately 0.5 mm into the right lateral ventricle (A-P: -0.20 mm; M-L: 1.75 mm; D-V: -3.00 mm from the surface of the brain). Each guide cannula was fixed into place with acrylic cement. In order to protect the guide cannula when not in use, a 30-gauge, stainless-steel syringe needle, crimped at one end and slightly longer than the guide cannula, was inserted into the guide cannula.

Following surgery, females were housed individually in standard wire-mesh cages in a colony room maintained on a reversed 12 hr light/12 hr dark cycle at approximately 21°C. Females were allowed free access to food and water. The placement of each guide cannula was tested 1 week before each
experiment by infusing 2 μg of angiotensin II into the right lateral ventricle of each rat. Because icv infusions of angiotensin II reliably induce thirst (Epstein, Fitzsimons, & Rolls, 1970; Lind & Johnson, 1982), only those females that displayed vigorous drinking within 5 min of infusion served as subjects.

Sexually active, adult Long-Evans hooded male rats, bred in our colony from stock originally obtained from Charles River Canada, Inc., Montreal, were used as stimulus males. All of these males were housed in groups of six in standard wire mesh group cages and maintained under colony conditions identical to those of the females.

**Drug Procedures**

Estradiol benzoate (EB) and progesterone (P) (Steraloids) were dissolved in peanut oil and injected sc in 0.1 ml of solvent. Angiotensin II (Bachem) was dissolved in a physiological saline solution at a concentration of 1 μg/μl. β-endorphin (Sigma), morphiceptin (Bachem), δ-receptor peptide (Bachem), and dynorphin 1-9 (Peninsula) were dissolved in a physiological saline solution to obtain concentrations of 10, 100, or 1000 ng/μl of solvent. All doses of these peptides were infused in 2 μl of solvent. Under control conditions, rats received an equivolume infusion of the physiological saline solution. All central infusions were made with an electrically driven infusion pump (Sage Instruments Model 3H1A) at a rate of 5 μl/min. Naloxone hydrochloride (a generous gift from Dr. V. Nicholson of duPont Pharmaceuticals) was dissolved in a physiological saline solution and administered sc at a
concentration of 10 mg/ml.

**Behavioural Testing**

Lordosis testing involved the presentation of each experimental female to a sexually vigorous stimulus male in a 29 x 45 cm Plexiglas testing chamber. The floor of the chamber was covered with 5 cm of San-i-cel bedding. Each female was placed with a male until 20 mounts with pelvic thrusting had occurred. Lordosis quotients were calculated as the percentage of mounts with pelvic thrusting that resulted in a lordosis posture. A moderate degree of sexual receptivity was induced in all experimental females by subcutaneous (sc) administration of 10 \( \mu \)g EB 48 hr and 250 \( \mu \)g P 4 hr before each test. All testing occurred during the middle third of the dark cycle in a room dimly lit by red lights.

In Experiments 1 through 4, the first group of experimental females (N=16) received infusions of either 0, 20, 200, or 2000 ng of each peptide at weekly intervals in a latinized fashion, such that all females were administered each dose of the peptide over a 4-week period in each experiment. Within each experiment, lordosis quotients were determined for each female 15, 30, 60, 90, and 120 min after infusion. In Experiment 5, the second group of experimental females (N=72) was assigned randomly to an effective dose of each peptide and received sc injections of either naloxone (10 mg/kg) or physiological saline 10 min before peptide infusion in a latinized fashion at weekly intervals. All experiments were separated by a 2-week period, during which an intervening angiotensin II test was conducted. Any female not displaying vigorous drinking was removed from the study and
replaced with a new experimental female.

In order to assess the behavioural specificity of the effect of each peptide on lordosis, other behavioural measures, such as the frequency of ambulation, grooming, and the degree of reactivity to a pencil touching the lumbar region of the spine, were recorded during the 5-min period before each lordosis test.

**Histological Analyses**

Guide cannula placements were verified for the group of experimental females used in the first four experiments and for those used in Experiment 5, by infusing a 5 μl quantity of black India ink into the right lateral ventricle of each rat once testing was complete. Rats were asphyxiated with CO₂ 20 min after ink infusion and were then perfused intracardially with saline followed by a 10 percent formalin solution. The brains were removed and dissected so that the lateral ventricles could be examined. Diffusion of ink throughout the ventricles was taken as verification of the correct placement of cannulae. Only the data from females with accurate cannula placements were subjected to statistical analyses.
Experiment 1:

Effect of \( \mu/\delta \) Receptor Activation with \( \beta \)-endorphin

\( \beta \)-endorphin appears to produce a dose-dependent dual effect on lordosis behaviour in female rats. Although the central infusion of low doses of \( \beta \)-endorphin, eg., 100 to 250 ng, has been reported to inhibit lordosis in OVX, steroid-primed rats (Sirinathsinghji, 1984; Sirinathsinghji et al., 1983; Wiesner & Moss, 1984; 1986a,b), preliminary experiments in our laboratory demonstrated that a higher dose of 2 \( \mu \)g facilitated lordosis behaviour in OVX, estrogen-primed rats 60 min after infusion into the lateral ventricles. However, several factors make it difficult to compare these results. For example, due to differences in route of administration, it is premature to conclude that the dual effect of \( \beta \)-endorphin represents the activation of different populations of opioid receptors that exert either an inhibitory or facilitatory action on lordosis behaviour. Differences in the steroid-priming regimens used could also contribute to the dual effect of \( \beta \)-endorphin on lordosis. For example, a steroid-priming regimen that induces maximal lordosis quotients would be expected to mask any facilitatory effect of \( \beta \)-endorphin. Finally, because \( \beta \)-endorphin binds to \( \mu \) and \( \delta \) receptors with relatively equal affinity, it is impossible to determine whether the dual effect represents dose- or time-dependent activity at either of these receptors.

A dose-response and time course analysis of the effect of \( \beta \)-endorphin on lordosis, using a common route of administration and an identical steroid-priming regimen for all animals in every dose condition, could provide a systematic way of
addressing whether the dual effect of β-endorphin is a function of one or more of the factors listed above. If route of administration and steroid-priming regimen can be ruled out as important variables in either the inhibition or facilitation of lordosis behaviour by β-endorphin, then an hypothesis concerning possible dose- or time-dependent activity at different opioid receptors would be strengthened considerably.

Accordingly, in Experiment 1, dose-response and time course analyses were conducted for the effect of β-endorphin on lordosis behaviour.

Method

In a latinized, repeated measures design, 16 experimental females received weekly treatments of 10 μg of EB followed 48 hr later by 250 μg of P. The administration of P was followed in 4 hr by icv infusions of either 0, 20, 200, or 2000 ng of β-endorphin. Lordosis behaviour was tested in each animal 15, 30, 60, 90, and 120 min after infusion. Data were evaluated using a 4x5x4 repeated measures analysis of variance to assess the main effects of dose, time course, order of presentation, and all interactions. For all statistically significant main effects and interactions, post-hoc comparisons were conducted using the Newman-Keuls method, p≤.05.

Results and Discussion

β-endorphin produced a dose dependent dual effect on lordosis behaviour as shown in Figure 1. The analysis of variance detected significant main effects for dose of β-endorphin, F(3,36) = 4.83, p<.006, and for time course, F(4,48) = 25.75, p<.0001. No significant main effect was found for order
Figure 1. Dose- and time-response curves for the effect of \( \beta \)-endorphin on lordosis behaviour in Experiment 1. Values represent mean lordosis quotients \( \pm \) standard errors.
of presentation. Post-hoc comparisons for the main effect of dose revealed that the 200 ng dose of β-endorphin significantly reduced lordosis quotients overall in contrast to the other three doses. Post-hoc comparisons for the main effect of time course revealed significant differences in overall lordosis quotients at 15, 30, and 60 min, but not at 90 or 120 min.

The analysis of variance also detected a significant interaction between dose and time course, $F(12, 144) = 4.36$, $p < .0001$. Subsequent post-hoc comparisons revealed that the 200 ng dose of β-endorphin significantly reduced lordosis quotients 15, 30, and 60 min after infusion, but not at 90 or 120 min. The 2000 ng dose of β-endorphin significantly facilitated lordosis quotients at 60 min, but did not produce significant differences from control values at any other time. The progressive rise in lordosis quotients throughout the testing period in animals that received control infusions of saline is a common effect of repeated lordosis testing (Larsson, Feder, & Komisaruk, 1974). However, this rise in lordosis quotients throughout the testing period did not reach statistical significance. All other interactions between dose and time course were also nonsignificant. Finally, none of the treatments with β-endorphin produced any noticeable changes in other behavioural measures such as reactivity, ambulation, or grooming. This suggests that the suppression or facilitation of lordosis behaviour by 200 or 2000 ng of β-endorphin respectively is not due to a general effect on motor activity.

The results of Experiment 1 are consistent with previous reports of an inhibitory effect of β-endorphin on lordosis
behaviour (Sirinathsinghji, 1984; Sirinathsinghji et al., 1983; Wiesner & Moss, 1984; 1986a,b). The time course of the inhibitory effect of 200 ng in Experiment 1 is similar to that reported by Wiesner and Moss (1984) following the infusion of 100 ng into the third ventricle. This suggests that the inhibition of lordosis produced by infusions of \( \beta \)-endorphin into either the lateral or third ventricles reflects a common interaction with opioid receptors. The facilitatory effect of 2000 ng of \( \beta \)-endorphin 60 min after infusion in Experiment 1 is also consistent with a previous observation in our laboratory that this dose facilitates lordosis behaviour in OVX, estrogen-primed rats. However, this effect is not consistent with recent data by Wiesner and Moss (1986b) showing an inhibitory effect of 2 \( \mu \)g of \( \beta \)-endorphin on lordosis behaviour. In that study, lordosis was tested 30 min after infusion of \( \beta \)-endorphin into the third ventricles of OVX rats primed with high doses of EB and P. Although the high degree of sexual receptivity induced by that steroid-priming regimen obviously precluded the ability to detect a facilitatory effect of \( \beta \)-endorphin in that study, it is more difficult to explain why Wiesner and Moss found an inhibition of lordosis with 2 \( \mu \)g of \( \beta \)-endorphin whereas the results of Experiment 1 clearly demonstrate a facilitation with this dose. Therefore, procedural differences between these studies need to be considered.

Comparable doses of \( \beta \)-endorphin or morphine, for example, produce a time dependent dual effect on continuous motor activity in an open field eg., ambulation and exploration, consisting of a short-term decrease followed by a prolonged
increase in activity (Babbini & Davis, 1972; Browne & Segal, 1980). It could be that higher doses of β-endorphin facilitate lordosis only in animals given repeated lordosis tests. β-endorphin may also interact with higher doses of P to inhibit lordosis. Such an interaction would be reminiscent of the inhibitory effect of p-Chlorophenylalanine (Gorzalka & Whalen, 1975) or cholecystokinin (Mendelson & Gorzalka, 1984) on lordosis behaviour. It may also be the case that progesterone alters the affinity or number of opioid receptors in the brain. It would be interesting to examine the effect of different steroid-priming regimens on opioid binding.

It is also impossible to rule out route of administration as a contributing factor. Although the inhibition of lordosis in the present experiment is consistent with previous reports from other investigators, who utilized different routes of administration, the facilitatory effect of 2 μg of β-endorphin may reflect a dose-dependent activation of opioid receptors near the lateral ventricles that serve to facilitate rather than inhibit lordosis behaviour. It should be noted, however, that Wiesner and Moss (1986b) failed to replicate the inhibition of lordosis reported by Sirinathsinghji (1984) following infusions of β-endorphin into the MCG. Thus, a precise determination of the factors that may contribute to these discrepant findings awaits further parametric research.
Experiment 2:

Effect of \( \mu \) Receptor Activation with Morphiceptin

Experiment 1 demonstrated a dose-dependent dual effect of \( \beta \)-endorphin on lordosis behaviour. However, because \( \beta \)-endorphin binds to \( \mu \) and \( \delta \) receptors with approximately equal affinity, it is impossible on the basis of Experiment 1 to determine the role of either of these opioid receptors in the inhibition or facilitation of lordosis by \( \beta \)-endorphin. A more appropriate analysis of opioid receptor mechanisms in lordosis behaviour requires the use of highly selective receptor ligands.

Morphiceptin (NH\(_2\)-Tyr-Pro-Phe-Pro-CO\(_2\)NH\(_2\)) is an opioid tetrapeptide derived from the milk protein \( \beta \)-casein. It has an affinity over 100 times greater for \( \mu \) than \( \delta \) or \( \kappa \) opioid receptors (Chang, Cuatrecasas, Wei, & Chang, 1982; Chang, Killian, Hazum, Cuatrecasas, & Chang, 1981) and produced a long-term, naloxone-reversible analgesia in mice following central administrations (Chang et al., 1982; Pasternak, Childers, & Snyder, 1980a,b; Zhang, Chang, & Pasternak, 1981). In order to assess the role of \( \mu \) receptor activation in lordosis behaviour, dose-response and time course analyses of the effect of morphiceptin were conducted.

**Method**

In a latinized, repeated-measures design, the 16 experimental females used in Experiment 1 again received weekly treatments of 10 \( \mu \)g EB followed in 48 hr by 250 \( \mu \)g of P. The administration of P was followed in 4 hr by icv infusions of either 0, 20, 200, or 2000 ng of morphiceptin. As in Experiment 1, the lordosis behaviour of each female was assessed 15, 30,
Results and Discussion

Morphiceptin produced a dose-dependent dual effect on lordosis behaviour as shown in Figure 2. The analysis of variance detected significant main effects of dose of morphiceptin, $F(3,36) = 29.15$, $p<.0001$, and of time course, $F(4,48) = 19.17$, $p<.0001$. As in Experiment 1, no significant main effect was found for order of presentation. Post-hoc comparisons for the main effect of dose revealed that the 20 ng dose of morphiceptin significantly reduced lordosis quotients overall in contrast to the other three doses. However, the two higher doses of 200 and 2000 ng significantly increased lordosis quotients overall in contrast to control infusions of saline. Although mean lordosis quotients at each time interval were higher in animals receiving 2000 ng than in those receiving 200 ng, this difference was not statistically significant. Post-hoc comparisons for the main effect of time course revealed a significant difference in overall lordosis quotients at 15 min but not at any other time. Overall lordosis quotients were significantly lower at 15 min in contrast to lordosis quotients at the other testing times.

The analysis of variance also detected a significant interaction between dose and time course, $F(12,144) = 6.14$, $p<.0001$. Subsequent post-hoc comparisons revealed that the 20 ng dose of morphiceptin significantly reduced lordosis quotients for the duration of the experiment in contrast to animals receiving saline infusions. Mean lordosis quotients for animals
Figure 2. Dose- and time-response curves for the effect of morphiceptin on lordosis behaviour in Experiment 2. Values represent mean lordosis quotients ± standard errors.
MORPHICEPTIN DOSE/TIME RESPONSE

Legend

- ▲ 0 ng
- X 20 ng
- □ 200 ng
- □ 2000 ng
receiving 20 ng did not differ significantly at 15, 30, or 60 min. However, at 90 and 120 min lordosis quotients had risen significantly from the previous three testing times, although lordosis quotients at 90 or 120 min were significantly lower than those of control animals. The 200 and 2000 ng doses of morphiceptin significantly facilitated lordosis quotients for the duration of the experiment in contrast to control animals. Significant differences in the degree of facilitation by these two doses were also detected at 15 and 30 min, but not at any other testing time. As in Experiment 1, the progressive rise in the mean lordosis quotients of saline treated control animals throughout the testing period did not reach statistical significance. All other interactions between dose and time course were nonsignificant. Finally, none of the treatments with morphiceptin produced any noticeable changes in the other behavioural measures, eg., reactivity, ambulation, or grooming. This suggests that the dual effect of morphiceptin is not due to a general effect of the peptide on motor activity.

It is difficult to explain the dual effect of morphiceptin in terms of its action on opioid receptors. Given the high degree of selectivity for \( \mu \) receptors, a linear dose-response function would be the most logical prediction and, indeed, the easiest to explain. The dual effect raises several possibilities. Morphiceptin has been shown to interact with both the high- (\( \mu_1 \)) and low- (\( \mu_2 \)) affinity configurations of the \( \mu \) receptor (Chang et al., 1982; Villiger, 1984; Zhang et al., 1981). Thus the inhibition of lordosis by 20 ng of morphiceptin could be due to the relatively selective activation of \( \mu_1 \),
receptors whereas the facilitatory effect of the 2 higher doses could be due to the activation of \( \mu_2 \) receptors or other opioid receptors. Although this hypothesis is tentative, there is evidence to suggest that \( \mu_1 \) and \( \mu_2 \) receptors mediate different, and in some cases opposite, effects of opioid drugs (Pasternak et al., 1983; Yonehara & Clouet, 1984).

Systemic injections of morphine, for example, result in analgesia, hypothermia, respiratory depression, bradycardia, sedation, catalepsy, increased release of prolactin and growth hormone, and a dual effect on dopamine turnover (Broderick, 1985; Pasternak et al., 1983; Yonehara & Clouet, 1984). Through the use of selective opioid receptor antagonists, Pasternak et al. have determined that supraspinal analgesia, prolactin release, hypothermia, catalepsy, and the decreased turnover of striatal dopamine, are produced by the agonist activation of \( \mu_1 \) receptors. Other effects of morphine, such as respiratory depression, bradycardia, and the increased turnover of striatal dopamine appear to involve either \( \mu_2 \) or \( \delta \) receptors. Consistent with this, the analgesic, but not the respiratory depressant, effect of morphine has been reversed by the \( \mu_1 \) antagonist naloxazone (Zhang et al., 1981). In order to determine whether the dual effect of morphiceptin on lordosis behaviour reflects a dose-dependent activation of \( \mu \) receptor subtypes, it is necessary first to rule out the possibility that morphiceptin produces either of these effects by binding to other opioid receptors. If the relatively selective activation of \( \delta \) or \( \kappa \) receptors has no effect on lordosis behaviour, then this hypothesis would be strengthened considerably.
**Experiment 3:**

Effect of δ Receptor Activation with δ-Receptor Peptide

Experiments 1 and 2 demonstrated a dose dependent dual effect on lordosis behaviour of β-endorphin and morphiceptin, respectively. The results of Experiment 2, however, have made it difficult to interpret the dual effect of β-endorphin as a function of the differential activation of μ and δ receptors. In light of the dual effect of morphiceptin, the effects of β-endorphin could reflect the differential activation of μ₁ and μ₂ receptors. It is therefore necessary to test the effect of a relatively selective δ receptor agonist on lordosis behaviour.

δ-receptor peptide (NH₂-D-Tyr-Ser-Gly-Phe-Leu-Thr-CONH₂) is a synthetic hexapeptide over 620 times more potent at δ receptors than at μ receptors, with no activity whatsoever at κ receptors (Gacel, Fournie-Zaluski, & Roques, 1980). Its relative potency and long duration of action at δ receptors occurs because of the novel arrangement of amino acids in the peptide, which greatly retards its degradation. The substitution of a D-Serine in the second position of the peptide inhibits the action of aminopeptidase. The addition of a Threonine residue at the C-terminal prevents the normally rapid cleavage of the Phe⁴-Leu⁵ bond by enkephalinase. In order to assess the role of δ-receptor activation in lordosis behaviour, dose-response and time course analyses were conducted for the effect of δ-receptor peptide.

**Method**

In a latinized, repeated-measures design, the 16 experimental females used in Experiments 1 and 2 again received weekly treatments of 10 μg EB followed in 48 hr by 250 μg of P.
The administration of P was followed in 4 hr by icv infusions of either 0, 20, 200, or 2000 ng of δ-receptor peptide. Lordosis behaviour was tested in each animal 15, 30, 60, 90, and 120 min after infusion. Data were evaluated statistically as in Experiment 1.

Results and Discussion

δ-receptor peptide produced a dose-dependent facilitation of lordosis behaviour as shown in Figure 3. The analysis of variance detected significant main effects for dose of δ-receptor peptide, \( F(3,36) = 25.16, p<.0001 \), and for time course, \( F(4,48) = 4.46, p<.004 \). As in Experiments 1 and 2, no significant main effect was found for order of presentation. Post-hoc comparisons for the main effect of dose revealed that 20, 200, and 2000 ng of δ-receptor peptide significantly increased mean lordosis quotients overall in contrast to control infusions of saline. No significant differences were detected among the three doses of δ-receptor peptide on overall lordosis quotients.

The analysis of variance also detected a significant interaction between dose and time course, \( F(12,144) = 7.56, p<.0001 \). Subsequent post-hoc comparisons revealed no differences among doses of δ-receptor peptide until 90 min, when lordosis quotients produced by 20 ng were significantly lower than those produced by 2000 ng. The increases in lordosis quotients produced by all three doses of δ-receptor peptide were significantly different from control values at 15, 30, 60, and 90 min. At 120 min, however, lordosis quotients of animals that received 20 or 200 ng were not significantly different from
Figure 3. Dose- and time-response curves for the effect of δ-receptor peptide on lordosis behaviour in Experiment 3. Values represent mean lordosis quotients ± standard errors.
DELTA-RECEPTOR PEPTIDE DOSE/TIME RESPONSE

Legend

- ▲ 0 ng
- X 20 ng
- □ 200 ng
- • 2000 ng

Mean lordosis quotient %

Minutes after injection
those of control animals. Although the 2000 ng dose produced a significant increase in lordosis quotients at 120 min, in contrast to the lordosis quotients of control animals, the magnitude of this effect was not significantly different from that of the two lower doses. Unlike the trends observed in Experiments 1 and 2, the progressive rise in the lordosis quotients of control animals throughout the testing period did reach statistical significance in Experiment 3. Lordosis quotients were significantly lower for control animals at 15 min than at any of the other testing times. All other interactions between dose and time course were nonsignificant. Finally, none of the treatments with δ-receptor peptide produced any noticeable changes in the other behavioural measures, eg., reactivity, ambulation, or grooming. This suggests that the facilitatory effect of δ-receptor peptide on lordosis behaviour is not due to a general effect of the peptide on motor activity.

The results of Experiment 3 indicate that the relatively selective activation of δ receptors with δ-receptor peptide serves to facilitate lordosis behaviour. In light of this, the facilitatory effect of β-endorphin or morphiceptin on lordosis cannot be attributed conclusively to agonist activity at μ₂ receptors. Higher doses of β-endorphin or morphiceptin could conceivably facilitate lordosis by an action on δ receptors. Resolution of this question awaits further research with selective μ and δ receptor antagonists.

It is interesting to speculate, however, on the role of endogenous activation of δ receptors in lordosis behaviour. DuPont et al. (1980) have shown that a single injection of
estradiol increases endogenous Met$^5$-enkephalin levels in the MCG. Similarly, Pfaff (1986, personal communication) has reported an increase in the synthesis of Proenkephalin in opioid neurons of the ventromedial hypothalamus following chronic estradiol administration. As mentioned earlier, lordosis behaviour is dependent upon the presence of estrogen, and chronic estrogen regimens facilitate lordosis in OVX rats. Thus, it is tempting to suggest that increases in endogenous enkephalin synthesis and release may disinhibit or facilitate lordosis behaviour. Although Sirinathsinghji (1984) reported that Met$^5$-enkephalin failed to influence lordosis behaviour 4 hr after infusion into the MCG of OVX rats primed with estrogen and progesterone, the results of Experiment 3, using a more selective and longer acting δ receptor agonist, support this suggestion.
Experiment 4:

Effect of κ Receptor Activation with Dynorphin 1-9

The role of κ receptors in lordosis behaviour has been controversial. Imura (1984) and Suda et al. (1986) reported a short-term facilitatory effect of leumorphin following icv infusions to OVX, estrogen-primed rats, whereas Sirinathsinghji (1984) reported no effect of dynorphin 1-17 4 hr after infusion to the MCG of OVX, estrogen-primed rats. However, because dynorphin 1-17 is short-acting, the results reported by Sirinathsinghji might have differed had lordosis testing been conducted closer to the time of administration.

Dynorphin 1-17 expresses its agonist action at κ receptors through its first metabolite, dynorphin 1-9 (NH₂-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-CONH₂). Dynorphin 1-9 is 16 times more potent at κ receptors than at δ receptors and over 20 times more potent at κ receptors than at µ receptors (Paterson et al., 1983). Because of its relative potency at κ receptors, and its diffuse localization throughout the pituitary, striatum, cortex, and cerebellum, dynorphin 1-9 has been classified as an endogenous ligand for κ receptors (Corbett et al., 1982). In order to assess the role of relatively selective κ receptor activation on lordosis behaviour, dose-response and time course analyses were conducted for the effect of dynorphin 1-9.

Method

In a latinized, repeated-measures design, the 16 experimental females used in Experiments 1, 2, and 3 again received weekly treatments of 10 μg EB followed in 48 hr by 250 μg of P. The administration of P was followed in 4 hr by icv
Infusions of either 0, 20, 200, or 2000 ng of dynorphin 1-9. Lordosis behaviour was tested in each animal 15, 30, 60, 90, and 120 min after infusion. Data were analysed as in Experiment 1.

Results and Discussion

Dynorphin 1-9 did not appear to affect lordosis behaviour, although the lordosis quotients of animals treated with dynorphin 1-9 appeared higher than those of control animals at 30 min (Figure 4). The analysis of variance detected a significant main effect for time course, $F(4,48) = 8.53, p<.05$, but not for dose of dynorphin 1-9 or order of presentation. Post-hoc comparisons for the main effect of time course revealed that the overall mean lordosis quotient at 15 min was significantly lower than the overall mean lordosis quotients at 90 and 120 min. However, no differences in overall mean lordosis quotients were detected between 15 and 30 min, nor between those at 30, 60, 90, or 120 min.

The analysis of variance also detected a significant interaction of dose and time course $F(12,144) = 3.82, p<.01$. However, subsequent post-hoc comparisons revealed no significant differences among doses of dynorphin 1-9 at any testing time. Although all three doses of dynorphin 1-9 appeared to facilitate lordosis at 30 min, in contrast to saline-treated control animals, the magnitude of this effect was not statistically significant. However, the facilitatory effect of the 2000 ng dose almost reached statistical significance in contrast to the effect of saline ($p<.10$). The progressive rise in the lordosis quotients of control animals throughout the testing period did reach statistical significance in this experiment. For control
Figure 4. Dose- and time-response curves for the effect of dynorphin 1-9 on lordosis behaviour in Experiment 4. Values represent mean lordosis quotients ± standard errors.
MEAN LORDOSIS QUOTIENT %

MINUTES AFTER INJECTION

Legend

DYNORPHIN 1-9 DOSE/TIME RESPONSE
animals, mean lordosis quotients at 15 and 30 min differed significantly from those at 60, 90, and 120 min. Finally, none of the treatments with dynorphin 1-9 produced any significant changes in the other behavioural measures.

In contrast to the robust facilitation produced by δ-receptor peptide or the two higher doses of morphiceptin, the facilitatory effect of dynorphin 1-9 was small and, more importantly, not statistically significant. Although it would be statistically correct to argue that dynorphin 1-9 had no effect on lordosis behaviour in Experiment 4, it would be premature to suggest that κ receptors play no role in the expression of lordosis behaviour based solely on the magnitude of the effect of dynorphin 1-9 in this experiment. The trend toward a significant facilitation of lordosis by the highest dose must be taken into consideration, especially in light of data showing a facilitatory effect of κ receptor activation with leumorphin (Imura, 1984; Suda et al., 1986).

The time course of the facilitatory effect of dynorphin 1-9, however, provides additional, albeit speculative evidence against a role of κ receptors in lordosis behaviour. Although dynorphin 1-9 acts as a preferential agonist at κ receptors, it displays a relatively lower affinity for δ receptors. Moreover, dynorphin 1-9 is transformed by the action of aminopeptidase into Leu⁵-enkephalin following cleavage of the Leu⁵-Arg⁶ bond (Griffiths & McDermott, 1984). Thus, the relative specificity of dynorphin 1-9 is shifted over time from κ to δ receptors. Given the results of Experiment 3, it is tempting to suggest that the delayed facilitatory effect of dynorphin 1-9 reflects a weak
action on δ receptors rather than on κ receptors. It is interesting to note that leumorphin is also transformed into Leu³-enkephalin by aminopeptidase. The facilitatory action of dynorphin 1-9 or leumorphin, therefore, may reflect the action of Leu³-enkephalin at δ receptors. It is not clear whether a larger dose of dynorphin 1-9 might significantly facilitate lordosis behaviour. Resolution of the question of receptor specificity awaits the use of either a selective δ or κ receptor antagonist in conjunction with dynorphin 1-9 or leumorphin.
Experiment 5:
Replication and Naloxone-Reversibility

In any study of the behavioural effects of opioid agonists, it is necessary to test whether those effects are reversible with an opioid antagonist. Reversibility indicates that the effect of a particular agonist reflects the specific activation of opioid receptors. When conducting experiments with novel compounds, or when acquiring results that are contrary to prediction, eg., a facilitatory effect of morphiceptin or δ-receptor peptide on lordosis behaviour, it is also advisable to test whether those results are reliable.

Therefore, in Experiment 5, naloxone or saline was administered in conjunction with icv infusions of β-endorphin, morphiceptin, or δ-receptor peptide to a new group of experimental females. The effective doses and testing time were chosen on the basis of results from Experiments 1 through 3.

Method

New experimental females were randomly assigned to one of the three peptide treatment groups (N = 24/group). Within each peptide treatment group, females were randomly assigned to a particular dose of the peptide in a between-subjects design. Females assigned to β-endorphin received either 0, 200, or 2000 ng of the peptide (n = 8/dose). Females assigned to morphiceptin received either 0, 20, or 2000 ng of the peptide (n = 8/dose). Females assigned to δ-receptor peptide received either 0 or 200 ng of the peptide (n = 12/group). Within each dose group, females received naloxone (10 mg/kg) or the saline vehicle at weekly intervals in a latinized, repeated-measures design.
Naloxone or saline was administered 10 min before peptide infusions. Lordosis testing of the subjects in all groups commenced 60 min after peptide infusion. The data were analysed using mixed-design analyses of variance for each peptide treatment group to assess the main effects of peptide dose, antagonist treatment, and the interaction of peptide dose with antagonist treatment. For all statistically significant main effects and interactions, the significance of pairwise comparisons was determined by the Newman-Keuls method, p<.05.

Results and Discussion

In saline-treated animals, β-endorphin and morphiceptin produced a dose-dependent dual effect on lordosis behaviour that was reversed by naloxone (Figures 5 and 6). δ-receptor peptide facilitated lordosis in saline-treated animals and this effect was also reversed with naloxone (Figure 7). The analysis of variance detected significant main effects for dose of β-endorphin, F(2,21) = 4.63, p<.02; dose of morphiceptin, F(2,21) = 12.62, p<.005; and dose of δ-receptor peptide, F(1,22) = 5.74, p<.02. Post-hoc comparisons for the main effect of β-endorphin revealed that the 200 ng dose significantly reduced the overall mean lordosis quotient compared to that of control animals. Although the overall mean lordosis quotient produced by the 2000 ng dose was higher than that produced by saline infusions, this difference was not statistically significant. Post-hoc comparisons for the main effect of morphiceptin revealed that the 20 ng dose significantly reduced, whereas the 2000 ng dose significantly increased, the overall mean lordosis quotient in contrast to that of control animals. Post-hoc comparisons for
Figure 5. Effect of naloxone (10 mg/kg) or saline on the display of lordosis behaviour following effective doses of β-endorphin in Experiment 5. Values represent mean lordosis quotients ± standard errors. Open bars: Saline; hatched bars: Naloxone.
Figure 6. Effect of naloxone (10 mg/kg) or saline on the display of lordosis behaviour following effective doses of morphiceptin in Experiment 5. Values represent mean lordosis quotients ± standard errors. Open bars: Saline; hatched bars: Naloxone.
Figure 7. Effect of naloxone (10 mg/kg) or saline on the display of lordosis behaviour following effective doses of δ-receptor peptide in Experiment 5. Values represent mean lordosis quotients ± standard errors. Open bars: Saline; hatched bars: Naloxone
Mean lordosis quotient %

Dose of delta receptor peptide (ng)

Legend:
- □ Saline
- □ Naloxone (10 mg/kg)
the main effect of δ-receptor peptide revealed that the 200 ng dose significantly increased the overall mean lordosis quotient in contrast to that of control animals.

A significant main effect of antagonist administration was detected only for δ-receptor peptide, F(1,22) = 21.11, p<.0002. Post-hoc comparisons revealed that animals receiving saline administrations, regardless of the dose of δ-receptor peptide, had an overall mean lordosis quotient significantly higher than animals receiving naloxone.

Significant interactions between peptide dose and antagonist administration were detected for β-endorphin, F(2,21) = 24.09, p<.0001; morphiceptin, F(2,21) = 17.80, p<.0001; and δ-receptor peptide, F(1,22) = 8.04, p<.009. Subsequent post-hoc comparisons revealed that administrations of naloxone significantly reversed the inhibition of lordosis produced by 200 ng of β-endorphin and by 20 ng of morphiceptin. Naloxone administration also significantly reversed the facilitation of lordosis produced by 2000 ng of morphiceptin and by 200 ng of δ-receptor peptide. Although animals that received the 2000 ng dose of β-endorphin displayed lordosis quotients significantly lower in conjunction with naloxone than with saline, the saline scores of these animals were not significantly different from those of control animals that received either naloxone or saline. Naloxone had no effect in animals that received control infusions of saline within each treatment group.

The results of Experiment 5 have replicated and extended the results of Experiments 1, 2, and 3 by demonstrating that the inhibitory or facilitatory effect of each peptide is reversible.
with naloxone. These results strongly support the conclusion that the inhibitory or facilitatory effect of \( \beta \)-endorphin, morphiceptin, or \( \delta \)-receptor peptide reflects the specific activation of opioid receptors. It is not clear why the highest dose of \( \beta \)-endorphin failed to facilitate lordosis behaviour significantly in contrast to saline-treated control animals in this experiment. However, the ability of naloxone to significantly reduce lordosis quotients in animals that received the highest dose of \( \beta \)-endorphin suggests that the 2000 ng dose may have exerted a facilitatory effect in those animals. The fact that the magnitude of this effect was not significantly different from the mean lordosis quotients of control animals may be due to differential baselines of lordosis behaviour within each dose group.

Finally, although infusions of naloxone to the MCG or the spinal cord have been reported to facilitate lordosis behaviour in OVX, steroid-primed rats (Sirinathsinghji, 1984; Wiesenfeld-Hallin & Sodersten, 1984), the inability of naloxone to affect the lordosis behaviour of control rats in Experiment 5 suggests that endogenous opioid systems do not exert a tonic inhibitory or facilitatory influence on lordosis in OVX rats primed with estrogen and progesterone. This finding is consistent with previous reports that peripheral administration of naloxone has no effect on lordosis behaviour in OVX, estrogen-primed rats (Wiesner & Moss, 1984).
General Discussion

The results of the present series of experiments have demonstrated that the relatively selective activation of central opioid receptors differentially affects lordosis behaviour in OVX, steroid-primed rats. Activation of the \( \mu/\delta \) receptor complex with \( \beta \)-endorphin in Experiment 1 resulted in a dose-dependent dual effect in which a low dose (200 ng) inhibited whereas a higher dose (2000 ng) facilitated lordosis behaviour. Similarly, activation of \( \mu \) receptors with morphiceptin in Experiment 2 produced a dose dependent dual effect in which the lowest dose (20 ng) inhibited whereas the two higher doses (200, 2000 ng) facilitated lordosis behaviour. Activation of \( \delta \) receptors with \( \delta \)-receptor peptide in Experiment 3 dose-dependently facilitated lordosis behaviour. Activation of \( \kappa \) receptors with dynorphin 1-9 in Experiment 4, however, did not have a significant effect on lordosis, although a nonsignificant trend toward facilitation appeared at the highest dose (2000 ng) 30 min after infusion. In Experiment 5, the dual effects of \( \beta \)-endorphin and morphiceptin, and the facilitatory effect of \( \delta \)-receptor peptide, were replicated in a different group of rats and were reversed with naloxone. This indicates that these effects are reliable and specific to the activation of opioid receptors in the present paradigm. The failure of naloxone alone to affect lordosis suggests that endogenous opioid systems do not exert a tonic action on lordosis behaviour.

Some of the results of the present experiments are contrary to earlier findings. For example, Wiesner and Moss (1986a,b) reported an inhibition of lordosis behaviour with 1, 2, and 4 \( \mu \)g
of β-endorphin. The results of Experiments 1 and 5 in the present thesis show a facilitation of lordosis with 2 μg of β-endorphin. Although the facilitation produced by this dose did not reach statistical significance in Experiment 5, the lack of an inhibitory effect is consistent with the observation in Experiment 1. Several methodological factors may have contributed to the different effects reported for higher doses of β-endorphin. Wiesner and Moss infused β-endorphin into the third ventricles of OVX rats primed with estrogen and a high dose of progesterone. In the present experiments, β-endorphin was infused into the lateral ventricles in OVX rats primed with estrogen and a low dose of progesterone. Although an interaction of β-endorphin with progesterone cannot be ruled out as a contributing factor, it seems rather unlikely given that the time course of the inhibitory effect of 200 ng in Experiment 1 was almost identical to that reported by Wiesner and Moss (1984) following the infusion of 100 ng into the third ventricle. This suggests that β-endorphin may inhibit lordosis in either of these paradigms by a common interaction with opioid receptors whereas the facilitatory or disinhibitory effect observed in Experiments 1 and 5 may reflect the interaction of higher doses of β-endorphin with a population of opioid receptors near the lateral ventricles which serves to facilitate lordosis behaviour. It is interesting to note that differences in the effect of serotonin on lordosis have been reported following lateral or third ventricular infusions (Wilson & Hunter, 1985). Another possibility is that higher doses of β-endorphin may facilitate lordosis in animals given repeated lordosis tests. In
the recent experiments by Wiesner and Moss (1986a,b), the effect of higher doses of β-endorphin was tested once, 30 min after infusion. Further parametric research is required to determine the precise factors that may contribute to the inconsistent findings for higher doses of β-endorphin.

The present experiments have also demonstrated a facilitatory effect of δ receptor activation on lordosis behaviour. However, this effect is not consistent with the lack of effect reported by Sirinathsinghji (1984) following infusion of the δ receptor agonist Met⁵-enkephalin to the MCG. Unlike δ-receptor peptide, Met⁵-enkephalin is a relatively short-acting peptide. Instead of testing the effect of Met⁵-enkephalin on lordosis soon after infusion into the MCG, Sirinathsinghji tested its effect 4 hr after infusion. The possibility that Met⁵-enkephalin had been cleaved by aminopeptidase into an inactive metabolite long before lordosis was tested in that experiment cannot be discounted. In contrast, the effect of δ-receptor peptide on lordosis was tested in Experiments 3 and 5 much sooner after infusion. Further research is required to determine whether the facilitatory effect of δ-receptor peptide is specific to its infusion into the lateral ventricles.

Although Sirinathsinghji (1984) also reported no effect of the κ receptor agonist dynorphin 1-17 on lordosis behaviour, Imura (1984) and Suda et al. (1986) have reported a short term facilitatory effect of leumorphin on lordosis. Both dynorphin 1-17 and leumorphin are relatively short acting κ agonists and both peptides contain the Leu⁵-enkephalin sequence at the N-terminus. Separating the Leu⁵-enkephalin sequence from either of
these peptides shifts their relative affinity from \( \kappa \) to \( \delta \) receptors. In Experiment 4, a trend toward a significant facilitation of lordosis by dynorphin 1-9 was observed 30 min after infusion of the highest dose (2000 ng). In light of the facilitatory effect observed after \( \delta \) receptor activation in Experiments 3 and 5, it is tempting to speculate that the facilitatory effect of either dynorphin 1-9 or leumorphin may reflect the agonist action of the Leu\(^5\)-enkephalin metabolite at \( \delta \) receptors.

Taken as a whole, the results of the present experiments suggest several possibilities about the role of opioid receptors in the central regulation of lordosis behaviour. As noted in the discussion of Experiment 2, morphiceptin has been shown to interact with both \( \mu_1 \) and \( \mu_2 \) receptor conformations. The dual effect of morphiceptin on lordosis may reflect the differential activation of these sites, such that binding to \( \mu_1 \) receptors inhibits lordosis whereas binding to \( \mu_2 \) receptors facilitates this behaviour. In a recent study, Pfaus, Pendleton, and Gorzalka (1986) showed that the selective, long acting \( \mu_1 \) receptor antagonist naloxazone reversed the inhibitory but not the facilitatory effect of morphiceptin on lordosis. Thus, it appears that \( \mu_1 \) receptors may play an exclusively inhibitory role in lordosis behaviour.

The dual effect of \( \beta \)-endorphin may be a result of the differential activation of \( \mu \) and \( \delta \) receptors. Recent evidence indicates that \( \mu \) and \( \delta \) receptors may be allosterically coupled in a mutually inhibitory manner, such that binding to one receptor inhibits the conformational expression of the other
(Rothman et al., 1985; Rothman & Westfall, 1982). Thus, the inhibition of lordosis produced by low doses of \( \beta \)-endorphin may be the result of the activation of \( \mu \), receptors whereas the facilitation or disinhibition observed with the 2 \( \mu \)g dose may reflect increased activity at \( \delta \) receptors which allosterically inhibits the binding of \( \beta \)-endorphin to \( \mu \), receptors. If this hypothesis proves correct, then specific brain areas rich in \( \mu \) receptors, eg., hypothalamus, thalamus, striatum, and brainstem, or \( \delta \) receptors, eg., frontal cortex, midbrain, and brainstem (Khachaturian et al., 1985; Zhang & Pasternak, 1980) would be implicated in the central regulation of lordosis by opioid drugs.

It is important to reiterate that the activation of \( \mu_2 \) receptors or \( \delta \) receptors with highly selective ligands produces several similar effects, eg., respiratory depression (Pasternak et al., 1983; Pasternak & Wood, 1986; Rothman et al., 1985). In fact, Rothman et al. (1985) have suggested on the basis of binding data that \( \mu_2 \) and \( \delta \) receptors may be the same receptor protein. Thus, agonist activity at \( \mu_2 \) or \( \delta \) receptors may serve a similar facilitatory or disinhibitory role in lordosis and may suppress the inhibition of lordosis produced by agonist activity at \( \mu_1 \) receptors through an allosteric mechanism. The use of selective opioid receptor antagonists in conjunction with central infusions of \( \beta \)-endorphin, morphiceptin, or \( \delta \)-receptor peptide should provide a useful method of testing this hypothesis.

The results of the present experiments also allow speculation concerning the role of endogenous opioids in
lordosis behaviour. Hormonal treatments that facilitate lordosis behaviour, e.g., chronic peripheral administrations of estrogen, are known to increase endogenous enkephalin levels in the midbrain and ventromedial hypothalamus (DuPont et al., 1980; Pfaff, 1986, personal communication). Treatment with estrogen and progesterone also depletes immunoreactive β-endorphin levels in hypothalamic tissue homogenates along with concurrent elevations of β-endorphin levels in plasma and pituitary tissue homogenates (Hulse & Coleman, 1984). Thus, endogenous opioids may act to inhibit or facilitate lordosis behaviour depending upon the brain area, receptor type, or hormonal state of the animal. However, it should be remembered that the failure of naloxone alone to affect lordosis behaviour in Experiment 5 strongly suggests that endogenous opioid systems were not tonically active during those tests.

Like any first step, the data collected in this thesis generate more questions than they answer. Certainly the effects reported for the opioid peptides used in the present experiments are specific to lordosis behaviour in female rats and cannot be used in a more general sense to make inferences about their effects in male rats or in other species. Likewise, the speculation concerning the role of opioid receptors presented above is specific to lordosis behaviour in female rats. The real value of the present experiments lies in the introduction of a new methodology. Clearly, the use of highly selective, long acting opioid receptor ligands provides a valuable tool for determining the function of opioid receptors in any behavioural analysis. Conducting a systematic analysis of the sexual effects
of selective ligands in discrete brain areas such as the MCG or
the mediobasal hypothalamus in female rats could more thoroughly
elucidate the role of opioid receptors in lordosis behaviour and
provide important data on opioid receptor control of
neuroendocrine function in female rats. A similar approach might
be taken to investigate the role of opioid receptors in the
sexual behaviour of male rats or other rodents. A major goal of
such research is to provide animal models of opioid effects on
sexual behaviour from which clinically-relevant information can
be derived for the treatment of opioid induced sexual
dysfunction. Although the data in the present thesis constitute
no more than a first step, they contribute to an already
expansive knowledge of opioid receptors and their role in
various aspects of behaviour.
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