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NICOTINIC CHOLINERGIC INFLUENCES ON SEXUAL RECEPTIVITY IN FEMALE RATS (Rattus Norvegicus)

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NICOTINIC CHOLINERGIC INFLUENCES ON SEXUAL RECEPTIVITY IN FEMALE RATS (*Rattus Norvegicus*)

by

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ABSTRACT

NICOTINIC CHOLINERGIC INFLUENCES ON SEXUAL RECEPTIVITY IN FEMALE RATS (Rattus Norvegicus)

By

David Raymond Weaver

Previous work by Clemens and co-workers has shown that intracerebral infusions of muscarinic agonists induce sexual receptivity (lordosis) in estrogen-primed ovariectomized (OVX) female rats. Conversely, intracerebral infusions of muscarinic antagonists disrupt receptivity in estrogen plus progesterone primed OVX rats. The purpose of this thesis was to examine the contribution of the nicotinic cholinergic receptor in the regulation of lordosis behavior.

In agreement with the earlier work of Fuxe, Everitt & Hokfelt (Pharmacol. Biochem. Behav. 7, 1977, 147-151), nicotine (50, 100 or 200 µg/kg) facilitated lordosis behavior in estrogen-primed OVX rats 5 minutes after intraperitoneal (i.p.) injection. Pretreatment with the nicotinic antagonist mecamylamine (MECA, 2.5 or 10 mg/kg, i.p.) completely prevented facilitation of lordosis by nicotine (150 µg/kg). Pretreatment with the muscarinic antagonist atropine (30 mg/kg) reduced but did not prevent the facilitation of lordosis by nicotine; 7.5 mg/kg atropine was ineffective. Intracerebroventricular (ICV) infusion of the cholinesterase inhibitor eserine (physostigmine) facilitated lordosis in estrogen-primed OVX rats (Dohanich, Barr, Witcher & Clemens, Physiol. Behav. 32, 1984,

1021-1026), and atropine pretreatment completely prevented this response. In the study reported here, MECA pretreatment (5 or 10 mg/kg, i.p.) reduced the eserine-induced facilitation of lordosis but did not prevent it completely. MECA did not disrupt hormone-induced receptivity when administered either systemically (5 or 10 mg/kg, i.p.) or ICV (5 or 10 µg/cannula, bilaterally).

In conclusion, pharmacological activation of nicotinic receptors can facilitate sexual receptivity, but endogenous nicotinic transmission appears to be relatively less important than muscarinic transmission in the regulation of sexual receptivity in female rats.

To Jan

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INTRODUCTION

Hormonal and behavioral cyclicity

Females of most mammalian species copulate only during a restricted portion of the ovarian cycle (see Morali & Beyer, 1979). In normal, gonadally intact female rats, there is cyclicity both in the levels of ovarian hormones in the plasma and in the occurrence of sexual receptivity. Removal of the ovaries virtually abolishes copulatory behavior by removing the source of these ovarian hormones (Young, 1961). In cycling female rats, estradiol levels in plasma increase slowly, reaching a peak approximately 12 hours prior to the onset of sexual receptivity during proestus (Butcher, Collins & Fugo, 1974). Progesterone levels, in contrast, increase rapidly and peak 3-4 hours before the onset of behavioral estrus (Butcher et al., 1974; Feder, Resko & Goy, 1968; Feder, Brown-Grant, Corker & Exley, 1969; Sodersten & Eneroth, 1981). The ovary normally produces this spike of progesterone, and acute ovariectomy prior to progesterone release prevents the induction of receptivity (Powers, 1970). Together, these studies have demonstrated that ovarian estrogen and progesterone are responsible for the induction of sexual receptivity in normal, gonadally intact female rats. Estrogen treatment induces sexual receptivity in ovariectomized female rats, but sequential treatment with estrogen followed by

progesterone is more effective (Boling & Blandau, 1939; Beach, 1942; see Young, 1961 for review). On the basis of hormone replacement studies as well, then, it is possible to conclude that sexual receptivity is hormone dependent, and the critical hormones are estrogen and progesterone.

Hormone-dependent sexual behaviors

During the period of behavioral estrus ("heat"), female rodents typically display certain behaviors. Aggression and scent marking are examples of hormone-dependent behaviors whose frequency of occurrence varies with the estrous cycle. These behaviors will not be discussed further, although it is recognized that they may influence the occurrence of specific copulatory behaviors.

Estrus female rodents are typically more attractive to males, more attracted to males, and are more likely to allow the male to copulate. Thus, feminine sexual behavior has been conceptually divided into attractivity, proceptivity, and receptivity (Beach, 1976). Behaviorally, the greater attraction of females in estrus towards males is evident by the occurrence of "proceptive" behaviors. Females rats in estrus (either naturally or induced by exogenous hormone treatment) spend more time in the vicinity of males, approach them more frequently, and display "hopping-and-darting" and "ear-wiggling," behaviors which serve to excite the male and draw his attention to the female.

Receptivity, or the behavior allowing the male to actually copulate, is frequently assessed by the occurrence of the lordosis response. The lordosis posture is assumed by estrus female rodents in response to mounting by the male; lordosis consists of arching of the back, elevation of the head and perineum, and lateral deflection of the tail (Komisaruk, 1974; Pfaff, 1980). Lordosis allows the male to achieve penile insertion (intromission) which is obviously necessary for intravaginal ejaculation. Sexual receptivity is synchronized with ovulation, as the developing follicles produce the hormones that induce sexual receptivity. As a result of this synchronization between sexual receptivity and ovulation, there is also synchronization in arrival of ova in the oviducts and sperm in the vagina. Hormone-induced sexual receptivity is thus critical to successful reproduction in rodents.

The relationship between gonadal hormones and lordosis behavior in rodents has been under experimental investigation since the 1920's. In those early studies, the necessary role of the ovary in female reproductive behavior was established, as was the ability of synthetic ovarian hormones to induce behavioral estrus in ovariectomized female rodents (for references, see review by Beach, 1981). In the years that have followed, attention has focused on the mechanisms of steroid hormone action and the precise molecular species of hormones involved in the induction of sexual receptivity. The major questions regarding hormonal

regulation of sexual behavior have become:

1) By what mechanisms do ovarian hormones act in the nervous system to induce sexual receptivity ?

2) In which neural sites do ovarian hormones act to induce sexual receptivity ?

The following literature review will summarize the current literature pertaining to these questions.

Mechanisms of steroid hormone action

Once in the systemic circulation (either after release from the ovary or after exogenous injection), steroid hormones are distributed throughout the body. Cells which have specific intracellular proteins (receptors) that bind a hormone are referred to as target cells for that hormone. Steroid hormones are lipophilic, and therefore they are believed to enter cells by passive diffusion. Within target cells, the steroid binds to "cytosolic" receptors (see paragraph on terminology below). Following activation (binding) of the receptor by the steroid, the steroid-receptor complex becomes more closely associated with the chromatin. The activated steroid-receptor complex is thought to bind to the chromatin and alter the pattern of messenger RNA production (transcription). In this way, steroid hormones can influence the pattern of proteins produced by a target cell (O'Malley & Means, 1974).

As indicated above, the term "cytosolic" receptors may

be misleading. Activated ["nuclear"] receptors are separated with the nuclear fraction of cell homogenates, and are therefore distinguished from the un-activated ["cytosolic"] receptors. While cytosolic receptors have frequently been assumed to be cytoplasmic, recent evidence indicates that both cytosolic and nuclear estrogen receptors reside in the nucleus in at least some tissues, and that the recovery of cytosolic receptors in the cytosolic fraction is merely an artifact of the homogenization process (King & Greene, 1984; Welshons, Lieberman & Gorski, 1984).

Within the nervous system, it is thought that gonadal hormones influence the pattern of neural activity in several ways (Beyer, Larsson & Cruz, 1979). Gonadal hormones may affect neural activity by direct effects on cell membranes (Kelly, Moss & Dudley, 1977a; Kelly, Moss, Dudley & Fawcett, 1977b; Towle & Sze, 1983), and after longer latencies by altering the pattern of proteins produced by neurons as mentioned above (McEwen, Davis, Parsons & Pfaff, 1979). Specific protein products induced or suppressed by steroid hormone treatment may include intrinsic proteins of the cell membrane (therefore influencing excitability), metabolic enzymes (influencing energy availability), mRNA and protein cleavage enzymes and kinases (influencing the form of other protein products of the cell), receptor proteins (both for neurotransmitters and for other steroid hormones), and neurotransmitter-related enzymes. Through the production or suppression of certain proteins, then, gonadal hormones can

alter the activity of complex neuronal populations as well as of individual neurons. In this way, gonadal hormones can regulate a variety of hormone-dependent behaviors.

The importance of hormone-induced protein synthesis in the induction of sexual behavior has been demonstrated by several workers using drugs which prevent protein synthesis (see Meisel & Pfaff, 1984, for critical review). Systemic treatment with the RNA synthesis inhibitor actinomycin-D interferes with estrogen priming if administered prior to, concurrent with or up to 12 hours after estrogen injection (Quadagno, Hough, Ochs, Renner & Bast, 1980; Quadagno, Shryne & Gorski, 1971; Terkel, Shryne & Gorski, 1973; Whalen, Gorzalka, DeBold, Quadagno, Ho & Hough, 1974); actinomycin-D does not appear to interfere with the synergistic effect of progesterone after a normal period of estrogen priming (Quadagno et al., 1971, 1971) Results with intracerebral application of actinomycin-D support these conclusions (Ho, Quadagno, Cooke & Gorski, 1973/4; Quadagno et al., 1971, 1980; Terkel et al., 1973; Whalen et al., 1974). Systemic injections of the protein synthesis inhibitor anisomycin interferes with both estrogen and progesterone induction of receptivity (Parsons, Rainbow, Pfaff & McEwen, 1982; Rainbow, Davis & McEwen, 1980a). When taken together, these and other studies (see Sites of Ovarian Hormone Action section) demonstrate that hormone-induced alterations in gene expression leading to

alterations in the pattern of protein synthesis are necessary for the induction of sexual receptivity. The apparently discrepant results with actinomycin failing to block progesterone-induced receptivity after estrogen priming has led to the suggestion that estrogen and progesterone act by slightly different mechanisms, e.g., progesterone may act primarily at the translational level, while estrogen may involve both transcription and translation. This possibility is supported by the shorter latency to progesterone effects on lordosis relative to estrogen (e.g., Glaser, Rubin & Barfield, 1983; Parsons, MacLusky, Krey, Pfaff & McEwen, 1980).

Approaches to the hormonal regulation of receptivity

Ovarian hormones act within specific sites in the central nervous system (discussed in the next section) to induce sexual receptivity. Attempts to understand the relationship between hormones and behavior at the neurotransmitter level have taken two general approaches, neurochemistry and psychopharmacology.

Using the neurochemical approach, one can administer hormonal treatments which are known to induce sexual receptivity and then examine a variety of neural sites for changes in neurochemical parameters. In this way it is possible to demonstrate hormone-induced alterations in neurotransmitter synthetic and degradatory enzymes, receptor levels and transmitter turnover rates. A great deal of

research has been conducted using this approach, but it is not possible by this approach alone to demonstrate that a given neurochemical change is actually related to the behavior being studied. For example, alterations in hypothalamic muscarinic receptors induced by estrogen have been described by several groups (Assivar, Egozi & Sokolovsky, 1981; Dohanich, Witcher, Weaver & Clemens, 1982; Meyers & Clemens, 1985; Rainbow, DeGroff, Luine & McEwen, 1980), but this change has been interpreted to be related to sexual behavior by some investigators and to gonadotropin secretion by others, depending on the interest of the authors. Furthermore, standard neurochemical assays involve grind-and-bind methods, which can obscure within-site heterogeneity in transmitter modulation by steroids. In this way, a behaviorally relevant alteration in neurochemical parameters can be overlooked because of "dilution" of the change in some neurons by surrounding tissue in which there is no alteration. The neurochemical approach can only provide supporting evidence for the involvement of a given transmitter in specific sites in regulating a given behavior. As a result, relatively little emphasis will be placed on this approach in this literature review.

The second approach to the hormone- neurotransmitter-behavior relationship is to administer drugs which alter neurotransmission and subsequently to determine their effect on behavior. This psychopharmacological approach has been

neurotransmitters on sexual behavior. This thesis is concerned with nicotinic cholinergic psychopharmacology and sexual behavior; it is therefore appropriate to review the literature on the psychopharmacology of sexual behavior. First, however, it is necessary to consider the neuroanatomical substrates important in the hormonal activation of sexual receptivity.

Sites of ovarian hormone action

Several techniques have been used to implicate the involvement of specific neural sites in hormone-activated reproductive behavior. Sites of hormone action in the regulation of sexual receptivity have been implicated by steroid autoradiography, electrophysiology (stimulation and recording), brain lesions, and intracerebral applications of hormones, protein synthesis inhibitors, neurotransmitters and other drugs. A description of the steroid-concentrating cells in the female rat brain follows as an introduction to the sites that may be important for hormone-activated behaviors. An overview of the proposed neural pathway for lordosis (which combines information from a variety of techniques) will then be presented. While intracerebral microinfusion of neurotransmitter-related drugs has been used in this and other laboratories to implicate specific intracranial sites in the regulation of sexual receptivity (Dohanich & Clemens, 1981), more recent evidence has indicated that this method is not always site-specific

(Clemens, Dohanich & Barr, 1983; Dohanich, Barr, Witcher & Clemens, 1984). Similarly, Meisel & Pfaff (1984) have proposed that spread of infused actinomycin-D from the preoptic area (POA) to the ventromedial hypothalamus (VMH) is responsible for the behavioral effectiveness of POA infusions in disrupting lordosis, as powdered actinomycin-D application to the POA is not effective. Due to reservations about the power of microinfusion techniques in implicating specific sites in the regulation of lordosis behavior, the psychopharmacology of sexual behavior will be discussed in a separate section.

Steroid autoradiography has been used to identify sites which concentrate ovarian hormones. In the rat brain, tritiated estradiol becomes concentrated most reliably in cells in the hypothalamus and limbic system following systemic injection into ovariectomized (OVX) adults (Pfaff & Keiner, 1973). Specifically, diencephalic sites which concentrate estradiol are located in the medial preoptic area, medial anterior hypothalamus, ventromedial and arcuate nuclei, and the ventral premammilary nucleus. In the telencephalon, the medial and cortical nuclei of the amygdala become densely labeled; the lateral septum, olfactory tubercle, bed nucleus of the stria terminalis, hippocampus, diagonal band of Broca, entorhinal cortex and prepyriform cortex are also reliably labeled, although less intensely. In the midbrain, the mesencephalic central gray

(MCG) also contains estradiol-concentrating cells. Labelled cells were observed elsewhere in the nervous system, but in general these cells were not labeled consistently from animal to animal and were not heavily labeled. Stumpf (1970) found essentially similar results, with the exception that some of the less intensely labeled areas of Pfaff & Keiner (1973) were considered to be unlabeled by Stumpf (e.g., entorhinal cortex). In addition, Stumpf noted labelling of the organum vasculosum of the lamina terminalis and the subfornical organ, which are circumventricular structures outside of the blood-brain barrier.

As discussed by Pfaff & Keiner (1973), many of the brain regions which are labeled following systemic injection of labeled estradiol are related to the regulation of gonadotropin secretion, mating behavior, or both. Unfortunately, it is impossible to tell which cells are involved in which functions from autoradiography. Lesion and hormone implant studies have implicated several of these areas in the regulation of sexual behavior. Furthermore, there are anatomical connections between many of these areas, which supports the idea of functionally related estrogen-concentrating "systems" in the rat brain.

Pfaff & Keiner (1973) and McEwen & Pfaff (1973) have described the pathways connecting estrogen-concentrating regions as comprising five groups: 1) several areas (prepyriform and entorhinal cortex, olfactory tubercle, medial and cortical amygdala) receive input from the

olfactory or accessory olfactory bulbs; 2) the septum, olfactory tubercle, and diagonal band of Broca are connected to each other and to the medial forebrain bundle; 3) the medial and cortical amygdala project via the stria terminalis to the bed nucleus of the stria terminalis, medial preoptic area, medial anterior hypothalamus, ventromedial nucleus, and the ventral premammilary nucleus; ventral hippocampal neurons project via the medial corticohypothalamic tract to the medial anterior hypothalamus and the arcuate nucleus; the hippocampus is reciprocally connected to septum and the diagonal band of Broca; and the bed nucleus of the stria terminalis and septum project to the medial preoptic area; 4) anterior hypothalamic and preoptic areas project caudally to the ventromedial nucleus; 5) the preoptic hypothalamic region connects to the MCG via periventricular fibers and the median forebrain bundle. Many of these areas, and connections between them, have been implicated by other methods as being important for the display of hormone-activated sexual receptivity.

As noted in the Hormonal and behavioral cyclicity section of this INTRODUCTION, estrogen followed by progesterone is the most effective hormonal treatment for the induction of sexual receptivity in rats. It should be obvious that sites which accumulate progesterone are potential sites for hormonal actions on behavior. Several authors have looked at the distribution of progesterone

binding sites in rat brain. Using the synthetic progestin R5020 as a ligand, for example, Warembourg (1978) has demonstrated a high level of progestin binding in the preoptic area, the mediobasal hypothalamus, and the anterior pituitary. The induction of progestin receptors is estrogen-dependent in these areas (Roy, MacLusky & McEwen, 1979). Whalen and Luttge (1971) have presented evidence that tritiated progesterone is also taken up by cells in the midbrain.

Thus, on the basis of data from a variety of techniques, some sites important for hormone-induced sexual receptivity have been identified. Additional anatomical studies have examined connections between these sites in an attempt to construct a neural pathway for lordosis behavior. The following paragraphs review the criteria for identifying sites, the proposed influence of some of these sites, and their connections. It should be realized that some portions of the "pathway" are still highly speculative.

Lordosis behavior occurs in response to stimulation by the male. The tactile information from the flanks and perineum of the female is carried by the pudendal nerves and ascends in the anterolateral columns of the spinal cord (Kow, Montgomery & Pfaff, 1977). The location of terminals of these ascending anterolateral fibers coincide with the location of neurons that respond to lordosis-relevant somatosensory stimuli (Malsbury, Kelley & Pfaff, 1972;

Mehler, 1969); these neurons are in the MCG and surrounding subtectal regions. Carrer (1978) has suggested that one of these other midbrain sites is the peripeduncular nucleus (PPN). It is possible, however, that the PPN also contains fibers or neurons which send fibers to the MCG.

On the efferent side of the neural circuit controlling the lordosis reflex, it is known that the motoneurons of the axial musculature receive input from reticulospinal neurons (Brink, Modianos & Pfaff, 1981). These reticulospinal neurons originate in the medullary reticulum and are involved in the control of lordosis (Modianos & Pfaff, 1976, 1979). Neurons in the MCG project to the medullary core, presumably modulating its activity (Sakuma & Pfaff, 1980a, 1980c). The circuitry necessary for execution of the lordosis reflex is therefore complete at the level of the midbrain in that afferent and efferent pathways converge in the MCG (Sakuma & Pfaff, 1979a; Pfaff, 1980). The MCG is thus thought to be an area of integration between the diencephalic estrogen-concentrating areas (which influence MCG activity via descending pathways as just discussed) and the sensory and motor pathways involved in the lordosis reflex (Pfaff, 1980). Lesions of the MCG also disrupt hormone-activated lordosis behavior, as would be expected if it is an interface between facilitative descending influences and the actual reflexive components of the neural circuitry for lordosis behavior (Sakuma & Pfaff, 1979b). Of particular note is the finding that MCG stimulation produces

a very short-latency facilitation of lordosis, and the facilitation disappears with cessation of the stimulation (Sakuma & Pfaff, 1979a). This may indicate (in support of the suggestion of Pfaff/Sakuma) that the MCG is a part of the "final common pathway" leading to the spinal mechanisms directly responsible for the lordosis reflex. In contradiction to the apparently consistent model presented by Pfaff and co-workers, Arendash & Gorski (1983) have recently reported that electrical stimulation of the MCG suppresses lordosis behavior. The reason for this difference in results is unclear.

The activity of MCG neurons is in turn influenced by estrogen directly and also by descending influences from the forebrain. In particular, the MCG receives input from the a variety of steroid-concentrating areas known to be involved in the regulation of lordosis behavior. Most notable among these are the preoptic area (POA) and the ventromedial nucleus (VMN) (Sakuma & Pfaff, 1980 b, c). The anatomical connections between the hypothalamus (VMN and POA) and MCG have been clearly established (Conrad & Pfaff, 1976a,b; Krieger, Conrad & Pfaff, 1979).

Integrity of the connections between the VMN and the midbrain are important for the expression of estrogen-plus-progesterone activated lordosis behavior (Lopez & Carrer, 1982; Edwards & Pfeifle, 1981). It has been proposed that the bed nucleus of the stria terminalis and the lateral amygdala are relay stations between the

peripeduncular nucleus (PPN) and VMN (Lopez & Carrer, 1982). The PPN may be a relay point for information ascending via a lateral pathway to the VMN; descending information also travels from VMN toward PPN in this same lateral pathway. Edwards & Pfeifle (1981) have shown that disruption of fibers that pass from the VMN through the peripeduncular region to the MCG produces deficits in hormone-activated lordosis behavior. Asymmetrical damage to this lateral pathway (parasagittal knife cut on one side, PPN region lesion on the other) also disrupted hormonal activation of lordosis. Monogue, Kow & Pfaff (1980) have also demonstrated that disruption of the connections between VMN and periaqueductal gray produces deficits in hormone-activated lordosis behavior.

Lesions in certain forebrain areas disrupt hormone-activated lordosis behavior, presumably by removing a facilitative input to the midbrain circuitry (e.g., ventromedial nucleus of the hypothalamus [VMN, Carrer, Asch & Aron, 1973; Kennedy, 1964; Mathews & Edwards, 1977; Mathews, Donovan, Hollingsworth, Hutson & Overstreet, 1983; Pfaff & Sakuma, 1979a; Sakuma & Pfaff, 1980c), the habenula [Modianos, Hitt & Flexman, 1974; Modianos, Hitt & Popolow, 1975], the anterodorsal hippocampus [Cameron, Gage, Hitt & Popolow, 1979] and the anterior portion of the medial amygdaloid nucleus [Masco & Carrer, 1980]. The facilitative role of these areas is also supported by the finding that electrical stimulation of the VMN (Pfaff & Sakuma, 1979b) or

medial amygdaloid nucleus (Masco & Carrer, 1980) will increase sexual receptivity. It has been proposed that the behavioral effect of stimulation of the VMN is due to a VMN stimulation-induced increase in excitability of MCG neurons as has been demonstrated electrophysiologically (Sakuma & Pfaff, 1980b,c). Consistent with this suggestion is the finding that stimulation of the MCG itself leads to a dramatic increase in lordosis reflex scores in estrogen-primed females (Sakuma & Pfaff, 1979a). Furthermore, MCG lesions disrupt lordosis behavior, whether activated by hormones alone or by electrical stimulation of the VMN following estrogen priming (Sakuma & Pfaff, 1979b).

On the basis of electophysiological evidence, it appears extremely unlikely that these forebrain areas involved in hormone-induced sexual receptivity (e.g., VMN) are involved directly in the reflex circuit (Pfaff & Sakuma, 1979a). Instead, these areas modulate the excitability of the midbrain lordosis reflex circuit. On the basis of a variety of studies, it has been suggested that "the role of the ventromedial nucleus of the hypothalamus can be described as a tonic estrogen-dependent facilitation of supraspinal mechanisms which control lordosis and are located more caudally in the brain stem" (Monogue, et al., 1980, p. 277).

The hormone-dependence of this facilitative input has been examined by other workers. Using the logic that a site which is involved in the regulation of sexual behavior

should also respond to hormone following restricted intracranial application, the hypothalamic VMN has been identified as a site of hormone action in the regulation of estrus behavior (see Barfield, Glaser, Rubin & Etgen, 1984, for review). Barfield & Chen (1977) and Rubin & Barfield (1980) demonstrated that estradiol implants in the VMN were effective in priming the animals so that they became responsive to the behavioral effects of systemically administered progesterone. This effect is specific for the VMN and these implants appear to expose only the mediobasal hypothalamus to estrogen, as revealed by autoradiography (Davis, McEwen & Pfaff, 1979; Davis, Krieger, Barfield, McEwen & Pfaff, 1982). Thus the VMN has been shown to be an important site of estrogen action.

Using the same methods, the VMN has also been shown to be an important site for progesterone action following estrogen priming. Progesterone implants in the mediobasal hypothalamus/VMN effectively activate sexual receptivity in estrogen-primed female rats (Powers, 1972; Rubin & Barfield, 1983b, 1984). As with estradiol, the spread of tritiated progesterone from the implant site was restricted to the VMN (Rubin & Barfield, 1983a). The VMN has also been indicated as a site important for the progesterone-induced sequential inhibition phenomenon (Rubin & Barfield, 1984).

Using a similar approach, Glaser & Barfield (1984) and Rainbow, McGinnis, Davis & McEwen (1982) demonstrated that application of the protein synthesis inhibitor anisomycin to

the VMN prevented the induction of receptivity by systemic estrogen plus progesterone treatment. These results demonstrate that the VMN is an important site of progesterone action in the induction of sexual receptivity, and that protein synthesis in the VMN is required for progesterone-induced sexual receptivity in estrogen-primed female rats.

Evidence concerning the behavioral effect of progesterone application to the midbrain is contradictory. Ross, Claybaugh, Clemens & Gorski (1971) reported that progesterone implanted into the mesencephalic reticular formation (MRF) facilitated lordosis behavior in estrogen-primed OVX rats. In this study, estrogen priming could be accomplished through systemic injection of estradiol benzoate (EB) or direct implantation of EB into the MRF. In females primed with systemical injections of EB, MRF application of either progesterone or EB effectively induced receptivity. These results indicate the MRF is a behaviorally important site of action for both estrogen and progesterone. It is interesting to note that VMH implants of progesterone were found to be ineffective (but see Barfield and others above). In contrast, others have found hormone treatments of the MRF to be behaviorally ineffective (Powers, 1972; Rubin & Barfield, 1983b, 1984). Furthermore, application of the protein synthesis inhibitor anisomycin to the interpeduncular area of the MRF did not prevent hormone-induced receptivity. These results, in conflict

with those of Ross et al., indicate that hormone action within the MRF is not critical for hormonal induction of receptivity.

It is unclear how hormone exposure to a single site, the VMN, can induce sexual receptivity. Certainly, hormone-induced sexual receptivity involves the VMN. It is still somewhat surprizing that activation of a single "modulatory" site can activate estrous behavior. The VMN must be a particularly critical site, as its influence on the MCG must overcome the absence in activation in other portions of the system.

In contrast to the facilitative role of the VMN and other sites discussed so far, an inhibitory role has been proposed for several other diencephalic and telencephalic areas. This proposal is based on the finding that lesions in these areas facilitate the expression of estrogen-activated lordosis. This ignores the possibility that a lesion may lead to reorganization instead of a simple loss of function (Clemens, 1978). Areas identified in this way include the medial preoptic area (MPOA, Powers & Valenstein, 1972), lateral septum-bed nucleus of the stria terminalis (Gorzalka & Gray, 1981; Nance, Shryne & Gorski, 1975; McGinnis & Gorski, 1980), olfactory bulbs (Lumia, Meisel & Sachs, 1981), and the posterior portion of the lateral amygdaloid nucleus (Masco & Carrer, 1980). Electrical stimulation in some of these areas suppressed hormone-activated receptivity

(septum, Zasorin, Malsbury & Pfaff, 1975; MPOA, Napoli, Powers & Valenstein, 1972; Sakuma & Pfaff, 1979b; lateral amygdaloid nucleus, Masco & Carrer, 1980), further indicating their inhibitory role. Finally, reversible "lesions" can be produced by blocking protein synthesis in localized brain areas. Infusions of cycloheximide into the lateral septum, cortical amygdala, or medial preoptic area produces facilitation of lordosis in estrogen-primed OVX rats (Renner, Bast, Purcell & Quadagno, 1981); crystalline application of anisomycin to the POA also facilitates lordosis (Meisel & Pfaff, 1983). As suggested by Meisel & Pfaff (1984), it appears that gonadal hormones may depress protein synthesis in some (lordosis-inhibiting) brain areas in order to bring about sexual receptivity. The role of the POA in sexual receptivity has therefore been described as an inhibitory modulator, rather than a primary effector, of steroid-induced sexual receptivity (Renner et al., 1981). This interpretation is similar to that of Pfaff & Sakuma (already described).

Psychopharmacology of lordosis behavior

Monoamines

The earliest reports of monoaminergic psychopharmacology and sexual behavior demonstrated that monoamines were involved in sexual receptivity, but the drugs used (monoamine oxidase inhibitors and reuptake inhibitors) were not selective for one or the other of the monoamines (serotonin, dopamine, norepinephrine, and epinephrine). It was nevertheless possible to conclude that elevation of brain monoamines through blocking reuptake or degradation reduced lordosis behavior in estrogen plus progesterone primed female rodents (Meyerson, 1964, 1966). The studies that have followed have tried to identify the contribution of individual monoamine transmitters involved in the regulation of sexual receptivity through the use of more selective pharmacological tools.

An inhibitory role of serotonin (5-hydroxytryptamine, or 5-HT) in the regulation of feminine sexual behavior was proposed over 20 years ago (Meyerson, 1964), but this suggestion remains controversial (Ahlenius, Engel, Eriksson, Modigh & Sodersten, 1975; Carter & Davis, 1977; Clemens, 1978; Crowley & Zemlan, 1981; Meyerson, Palis & Sietnieks, 1979). Serotonergic transmission has been manipulated at the level of synthesis, storage, release, receptors, and degradation in attempts to more clearly define the role of serotonergic transmission in sexual receptivity.

Lordosis behavior is facilitated in estrogen-primed female rats by systemic injection of the serotonin synthesis inhibitor, para-chlorophenylalanine (pCPA; Ahlenius et al., 1975; Everitt, Fuxe & Hokfelt, 1974, 1975a; Everitt, Fuxe, Hokfelt & Jonsson, 1975b, 1975c; Meyerson & Lewander, 1970; Zemlan, Ward, Crowley & Margules, 1973. This effect of pCPA may be independent of its action on central serotonergic neurotransmission, however. PCPA is converted to pCPEA (p-chlorophenylethylamine); pCPEA produces a transitory depletion of catecholamines and directly stimulates lordosis behavior (Wilson, Bonney, Everard, Parrott & Wise, 1982). The depletion of catecholamines following pCPA treatment correlates with the short-latency of pCPA to facilitate lordosis (Ahlenius, Engel, Eriksson, Modigh & Sodersten, 1972) and not with the long latency required for serotonin depletion. Furthermore, benserazide, which prevents conversion of pCPA to pCPEA, prevents both pCPA-induced receptivity and the pCPA-induced depletion of NE, while not preventing 5-HIAA depletion (Wilson et al., 1982). It has been suggested that pCPEA also causes a brief release of 5-HT, and that this is responsible for the short-term facilitation of lordosis by pCPA (Wilson et al., 1982).

In addition to facilitating lordosis behavior, pCPA has been reported to decrease lordosis responding in EB plus P primed sexually receptive females when given repeatedly or at longer intervals (> 24 hours) before priming (Emery &

Larsson, 1979; Gorzalka & Whalen, 1975; Segal & Whalen, 1970; Singer, 1972). This type of regimen is most effective in reducing brain 5-HT levels. Wilson et al. (1982) and others have suggested that serotonin has biphasic effects on sexual behavior: the initial increase in serotonin release facilitates lordosis behavior, while sustained elevation or depletion inhibits lordosis.

To further complicate matters, there is evidence that pCPA may stimulate the release of progesterone from the adrenal gland (Wilson et al., 1982). Thus, the adrenal-derived progesterone may be responsible for the pCPA-induced facilitation of lordosis. Adrenalectomy should then prevent the PCPA facilitation of lordosis, and this has been observed by some (Eriksson & Soderstem, 1973; Gorzalka & Whalen, 1975) but not all (Everitt et al., 1974, 1975b, 1975c; Zemlan et al., 1973) investigators.

Thus, studies of pCPA and sexual receptivity have indicated that pCPA can deplete serotonin, deplete NE and DA briefly, facilitate lordosis with an intermediate latency (1-8 hr), and inhibit lordosis with long latency (> 24 hours). Adrenal progesterone may also provide a non-neurochemical explanation for the facilitative influence of pCPA on lordosis behavior.

Results with other depletors of 5-HT have been no less confusing. The drug pCA (p-chloroamphetamine) is both a depletor of serotonin in the long-term and an indirect short-term serotonin agonist because it causes release of

5-HT. Lordosis was inhibited by pCA 30 minutes after injection in female rats made receptive with EB plus P priming (Yamanouchi, Watanabe, Okada & Arai, 1982). Other investigators have found an inhibitory effect of pCA in estrogen-plus-progesterone primed females, while it was without effect in animals primed with estrogen alone (Sodersten, Berge & Hole, 1978; Zemlan, Trulson, Howell & Hoebel, 1977).

Chemically-induced 5-HT denervation of the hypothalamus by injection of the neurotoxin 5,7-DHT into the medial mesencephalic 5-HT bundles facilitates lordosis in estrogen-primed females (Everitt, Fuxe & Jonsson, 1975d). This facilitation was weak, however, and was observed only 100 days after lesion (and not at some earlier time points after lesion). In another study (Sodersten et al., 1978), 5,7-DHT chemical lesions were ineffective in facilitating lordosis. A more recent study (Luine, Frankfurt, Rainbow, Biegon & Azitia, 1983) has demonstrated the "rapid, dramatic and reliable increase in lordosis behavior when 5,7-DHT is applied directly within the hypothalamus". In this study, females with 5,7-DHT lesions were more receptive following EB priming than the control animals in tests 9 to 14 days post-lesion. These authors suggested that the lesions in the other studies may have been ineffective because they spared an important midbrain-hypothalamic serotonergic projection, or alternatively that the important serotonergic projection is intrahypothalamic. Regardless of the reason

for the differences in results between these studies, interpretation of these results is difficult. While 5,7-DHT lesions may facilitate lordosis behavior by removing an inhibitory serotonergic influence, it is also possible that these lesions induce denervation supersensitivity to 5-HT. This latter interpretation would indicate a facilitative role of 5-HT; receptor assay data are necessary to rule out this possibility. Luine et al. examined imipramine binding to measure 5-HT terminals; while presynaptic terminals were reduced, these authors made no measurements of postsynaptic receptor levels.

Using 5-HT receptor agonists, both stimulatory and inhibitory effects on lordosis have been reported, and the effect seems to be dose-related (see Crowley and Zemlan, 1981). For example, in estrogen-primed female rats, LSD (5-20 ug/kg) facilitated lordosis behavior, while low and high doses (1 and 40 µg/kg, respectively) were ineffective (see Everitt et al., 1975c). In estrogen-progesterone primed receptive females, however, 5-HT agonists such as LSD, psilocybin, dimethyltryptamine, p-chloroamphetamine, alpha methyl tryptamine and fenfluramine suppress lordosis behavior (Eliasson & Meyerson, 1977; Espino, Sano & wade, 1975; Everitt & Fuxe, 1977b; Everitt et al., 1974, 1975b, 1975c; Michanek & Meyerson, 1977; Yamanouchi, Watanabe, Okada & Arai, 1982). It has been suggested that low doses of agonist preferentially activate the presynaptic autoreceptors leading to a decrease in 5-HT release (and

facilitation of lordosis), while higher doses activate postsynaptic receptors (and inhibition of lordosis predominates).

Intracerebral infusion of 5-HT itself was ineffective in facilitating lordosis in estrone-primed female rats, but 5-HT did inhibit lordosis in moderately receptive females primed with a higher dose of estrone (Foreman & Moss, 1978b). The inability to produce facilitation of lordosis with 5-HT apparently contradicts the proposal by Wilson et al. (1982), that serotonin is necessary for lordosis. This contradiction results only if one assumes that the infusions by Foreman & Moss into MPOA and arcuate-ventromedial hypothalamus definitely exposed the critical neurons to 5-HT, and that the single dose of 5-HT infused produced the proper drug concentration at these neurons for facilitation. These assumptions are not warranted on the basis of currently available evidence.

Disruption of serotonergic transmission with the receptor antagonists methysergide and cinanserin facilitated lordosis, and the facilitation is independent of the adrenal gland (Everitt et al., 1974, 1975b; Foreman & Moss, 1978b; Henrik & Gerall, 1976; Ward, Crowley & Zemlan, 1975; Zemlan et al.).

In summary, there is evidence, albeit somewhat confusing and contradictory, to support the proposal that serotonin inhibits feminine sexual behavior. The data are also consistent in demonstrating that low doses of agonists

facilitate lordosis. This has led to the suggestion that there is selective activation of autoreceptors at low agonist doses, which reduces endogenous 5-HT and causes an increase in lordosis behavior. The multiple effects of some drugs that have been used (e.g., pCPA) has also added to the confusion. The possibility that adrenal steroids mediate the serotoninergic facilitation of lordosis has been largely excluded in several paradigms, especially in the case of antagonists.

Norepinephrine (NE) has been suggested as a transmitter that facilitates lordosis behavior (Everitt et al., 1975b, 1975c), but the role for adrenergic transmission is nevertheless unclear (Crowley & Zemlan, 1981). Some of the confusion may be due to the multiple receptor types for NE and epinephrine, and the varying selectivity of drugs for one or the other of these types of sites. In addition, several of the drugs used to study the adrenergic regulation of sexual receptivity also affect the other monoamines, dopamine and serotonin. Thus, drug studies using alpha-methyl-para-tyrosine (a tyrosine hydroxylase inhibitor), amphetamines (which release catecholamines) and reserpine (which disrupts reuptake) are not as selective as is necessary for a detailed pharmacological analysis. Similarly, results discussed earlier with pCPA and pCPEA suggest that reduction in hypothalamic NE and/or DA may be

involved in the facilitation of lordosis by these drugs (Wilson et al., 1982), but it is difficult to separate these effects from those on serotonin systems. The approach of temporally correlating the neurochemical effects of a drug with its behavioral effects and proposing a specific role for a given transmitter on that basis is weak, especially for drugs with multiple actions.

Studies using NE itself have also yielded conflicting results regarding the regulatory role of NE on sexual receptivity. Third ventricular infusion of NE reduced lordosis behavior in OVX, EB plus P primed females as well as in EB + pCPA treated females (Wilson et al., 1982). Caldwell (1983) has also reported that infusion of NE into the MPOA inhibited lordosis behavior in EB plus P primed receptive female rats, and this inhibition was reversed by simultaneous infusion of the alpha-2 adrenergic blocker yohimbine (but not by the alpha-1 antagonist phentolamine).

Clonidine (an alpha-2 agonist) and epinephrine were also found to inhibit lordosis in Caldwell's (1983) study. Clonidine had previously been reported to inhibit lordosis with short latency following intracerebral infusion (Davis & Kohl, 1977). Foreman & Moss (1978a) also found clonidine to inhibit lordosis. The clonidine (and NE) suppression of lordosis may be due to activation of presynaptic alpha-2 receptors, as the clonidine effect was blocked by yohimbine, a presynaptic (alpha-2) blocker, but not by phenoxybenzamine, a postsynaptic (alpha-1) blocker.

Furthermore, Everitt et al., (1975b, 1975c) reported that the alpha-2 blockers yohimbine and piperoxane facilitated lordosis. Thus it appears that activation of alpha-2 receptors suppresses lordosis behavior, while alpha-2 blockade facilitates lordosis. The suggestion that the alpha-2 sites mediating the adrenergic inhibition of lordosis are presynaptic has been challenged, as not all alpha-2 receptors are presynaptic (see Caldwell, 1983, for discussion).

If the lordosis-suppressing effect of alpha-2 receptor acivation is due to actions at a presynaptic autoreceptor, then this indicates that a reduction in NE outflow suppresses lordosis, e.g., lordosis is facilitated by NE. Caldwell (1983) examined this possibility directly, and found NE did not facilitate lordosis except at a high dose (20 ug) which he considered debilitating to the animals. As already noted, Wilson et al., (1982) also found an inhibitory effect of NE infusion on hormone-activated lordosis behavior. Thus, from these studies the role of NE appears to be inhibitory rather than facilitative.

In another study, however, NE microinfusion into the POA or VMH facilitated lordosis in estrogen-primed female rats 105 minutes after infusion (Foreman & Moss, 1978a). This facilitative effect of NE was mimicked by the beta receptor agonist isoproterenol. While the proposal of a facilitative role of beta receptor activation is strongly supported by the finding that infusion of the beta blocker propranolol

reduced lordosis in estrogen-primed females, it also directly contradicts the earlier finding that beta blockers facilitate lordosis (Ward et al., 1975). The behavioral effects of adrenergic drugs observed by Foreman & Moss had a long latency (105 minutes to peak effect) and required repeated matings to occur. These latter factors raise the possibility of adrenal steroid mediation of this effect. In general, paradigm-dependent and long-latency effects of this sort cannot reasonably be attributed to a neurotransmitter-dependent mechanism. The contradictions between experimenters, who each use their own hormone priming regimens, drug delivery locations and routes, and behavioral testing schedules are extremely difficult to resolve. Finally, some experimenal regimens which use repeated testing and long latencies to testing do not adequately control for the possibility of release of adrenal steroids, which could potentially facilitate lordosis behavior in estogen-primed females.

In summary, the use of receptor subtype-selective agonists and antagonists has led to the proposal that activation of alpha-2 receptors inhibits lordosis. Other suggestions have also been made, but the role of the alpha-2 receptor seems to be the only point on which there is some agreement. It can be disputed whether these alpha-2 receptors are presynaptic, resulting in a decrease in NE outflow, or postsynaptic, resulting in NE-like agonistic actions. As a result, there is still confusion over the

role of NE, despite the fact that the action of one class of receptor agonists is agreed upon. Every possible combination of alpha-1, alpha-2, and beta receptors with inhibitory and facilitative roles has been proposed. The role of adrenergic transmission in the regulation of sexual receptivity is obviously unclear.

Dopamine (DA) has been suggested to be inhibitory to sexual receptivity. This suggestion is supported by the finding that DA agonists suppress lordosis, while DA antagonists activate lordosis (citations below).

DA receptor stimulants (e.g., apomorphine, ET 495) suppress lordosis in estrogen plus progesterone primed female rats (Everitt et al., 1974, 1975b, 1975c; Meyerson, Carrer & Eliasson, 1974). Amphetamine, a releaser of catecholamines, also suppresses lordosis, although this effect is not clearly related to DA alone (Eliasson, Michanek & Meyerson, 1972; Meyerson, 1968; Michanek & Meyerson, 1977). The effects of agonists have been described as biphasic or dose-dependent, however: low doses of agonist facilitate lordosis in estrogen primed female rats, and higher doses disrupt receptivity in estrogen plus progesterone primed females (Everitt & Fuxe, 1977a; Hamburger-Bar & Rigter, 1975). This dose-dependency is thought to be due to the activation of presynaptic "autoreceptors" by low doses of agonist, which results in decreased DA release and hence a facilitation of lordosis.

Higher doses are thought to act directly at the postsynaptic receptors to inhibit lordosis. Little has been done to rigorously test this site-of-action hypothesis for the dose-dependent effects of dopaminergic drugs.

Consistent with the proposed inhibitory role of DA are studies demonstrating that DA antagonists (pimozide, spiroperidol) activate lordosis in estrogen-primed female rats (Everitt et al., 1974, 1975b, 1975c). The finding that intraventricular infusion of the catecholamine neurotoxin, 6-hydroxydopamine, increased lordosis behavior is also consistent with the proposed inhibitory role of DA (Caggiula, Herndon, Scalon, Greenstone, Bradshaw & Sharp, 1979; Herndon, Caggiula, Sharp, Ellis & Redgate, 1978). This technique is not precise or selective enough to allow one to conclude with certainty that the observed behavioral effect is due to destruction of DA neurons alone, however.

Using intrahypothalamic (MPOA or ARC-VMH) infusion of DA or the DA agonist apomorphine, Foreman & Moss (1979) demonstrated a facilitation of lordosis in estrone-primed OVX female rats. Infusion of the DA antagonists haloperidol and alpha-flupenthixol to these same areas depressed lordosis behavior in females made receptive with a high dose of estrone. This study, which demonstrates a long-latency facilitative role of DA on lordosis behavior, may differ from others because of site-specificity in the response; e.g., the results of systemic treatment may differ from intrahypothalamic infusion. It is also impossible to

compare the systemic and intracerebral doses to see if the infusions produce "low" doses of agonist which would act preferentially at the autoreceptors. These authors have proposed that DA facilitates lordosis, and that DA does so by stimulating luteinizing hormone-releasing hormone (LHRH) release. (The psychopharmacology of LHRH will be discussed later in this section.) LHRH is in turn dependent upon dopaminergic neurons for its effectiveness, suggesting a positive feedback loop relationship. It seems unlikely that the facilitative effect of DA on lordosis could be mediated by LHRH, as LHRH takes several hours longer than DA to produce its behavioral effects in the hands of Moss and co-workers. The effects of LHRH as described by these workers require a long latency and repeated testing, although it has been shown that they are independent of the adrenal and pituitary glands. Other workers (e.g., Pfaff, see LHRH discussion, below) see more rapid, more reliable and more robust facilitation of lordosis by LHRH.

In summary, DA and DA agonists appear to inhibit lordosis behavior in most studies, although the dose-response is biphasic. Some studies cited in suppport of an inhibitory role of DA are not selective enough for DA neurons or receptors, although the more recent trend seems to be toward improving this selectivity through the use of more selective drugs. DA antagonists facilitate lordosis.

Gamma-aminobutyric acid (GABA)

McGinnis, Gordon & Gorski (1980) examined the effects of alterations in GABA transmission on hormone-dependent lordosis behavior. Picrotoxin, a GABA antagonist, and hydrazinopropionic acid (HPA), a drug which elevates endogenous GABA levels by inhibiting the degradatory enzyme, GABA transaminase, were used. Picrotoxin infusion into the substantia nigra reduced receptivity in EB-primed females with septal lesions. Intranigral HPA facilitated lordosis in EB-primed sham animals. These results are consistent with the suggestion that GABA facilitates lordosis in OVX female rats. Biochemical evidence presented in the same paper indicated that this effect may be mediated by dopamine: exogenous intranigral GABA reduces turnover of dopamine, and DA (from nigra and other mesencephalic areas) inhibits lordosis (see above), so GABA facilitates lordosis by reducing DA activity. The increased behavioral sensitivity of animals with septal lesions to estrogen may be due to lesion-induced depression of DA levels.

Luteinizing hormone-releasing hormone

The hypothalamic neuropeptide luteinizing hormone releasing hormone (LHRH; also called gonadotropin releasing hormone, or GnRH) has been shown to facilitate lordosis behavior following systemic injection in estrogen-primed OVX rats (Moss & McCann, 1973; Pfaff, 1973). In these first

studies, this estrus-facilitating action was shown to be independent of the pituitary. More recent studies have demonstrated that intracranial application of LHRH also facilitates lordosis behavior with short latency, with the most effective sites being the ventromedial-arcuate area of the mediobasal hypothalamus, the preoptic-anterior hypothalamic area, and the MCG (Moss & Foreman, 1976; Sakuma & Pfaff, 1980, 1983; Rodriguez-Sierra & Komisaruk, 1982; Riskind & Moss, 1979, 1983). The spinal cord has also been indicated as a potential site of LHRH action (Sirinathsinghji, 1982). Considering the recent evidence from this laboratory concerning the possible behavioral effects resulting from movement of infused substances up the cannula tracks (Dohanich et al., 1984), it appears that site specificity of response may not be a realistic expectation.

Independence of the lordosis-facilitating effect of LHRH and regulation of the pituitary have been clearly demonstrated not only by the use of hypophysectomy, but also by examining the effectiveness of LHRH analogs to influence sexual receptivity and LH secretion. Studies of this type have clearly indicated selectivity differences between the receptors regulating LHRH effects on reproductive behavior and LH secretion (Kastin, Coy, Schally & Zadina, 1980; Zadina, Kastin, Fabre & Coy, 1981; Sakuma & Pfaff, 1983; Dudley, Vale, Rivier & Moss, 1981). LHRH antisera have also been used, but the results have not been consistent.

Infusion of LHRH antiserum disrupts estrogen-activated lordosis following infusion into the MCG (Sakuma & Pfaff, 1980d, 1983) and the third ventricle (Kozowski & Hostetter, 1978), while third ventricle infusions into naturally cycling proestrus females were ineffective (Cooper, Seppala & Linnoila, 1984), perhaps because of synergism between E and P in the intact females. Intracerebral application of LHRH antisera into the ventromedial-arcuate region (Dudley et al., 1981) and the medial preoptic area (Cooper et al., 1984) were ineffective in disrupting estrogen-activated lordosis behavior, perhaps indicating that LHRH neuromodulation/ neurotransmission in these sites is not as important as it is in the midbrain for the expression of lordosis behavior. Alternatively, variation between the antisera used could be responsible for the discrepancy between sites examined in different laboratories.

Oxytocin and vasopressin

Oxytocin and vasopressin are peptides produced primarily by cells in the supraoptic and paraventricular nuclei of the hypothalamus. These neurons project to the posterior pituitary and release their peptides into the circulation there. Additional projections and sites of origin of oxytocinergic and vasopressinergic cells within the brain have recently been described (Caffe & van Leeuwan, 1983; Sofroniew & Weindl, 1981; Van Leeuwan & Caffe, 1983),

raising the possibility that these peptides also function as neuromodulators or neurotransmitters in the central nervous system. Accordingly, these peptides have recently been investigated for effects on hormone-dependent sexual behavior.

Intracerebral oxytocin treatment facilitates sexual receptivity in estrogen-primed female rats (Caldwell, Pedersen & Prange, 1984). Due to the fairly high level of EB priming used, however, the control animals also showed an increase in lordosis over time after infusion. While the oxytocin treatment increased lordosis above the level seen in the vehicle controls, the possible effect of repeated testing was not controlled. It is nevertheless quite apparent from this study that oxytocin can facilitate lordosis behavior.

Sodersten, Henning, Melin & Ludin (1983) have recently shown that ICV injections of vasopressin (1 to 10 ng) can inhibit sexual receptivity in receptive rats. This effect was independent of alterations in blood pressure and its specificity was demonstrated by blocking it with pretreatment with antiserum to arginine vasopressin.

Other peptides

Several other hypothalamic and pituitary peptides have also been examined for their influence on sexual receptivity. Systemic injection of alpha-MSH has been reported to both potentiate and disrupt lordosis behavior in

estrogen plus progesterone primed female rats, with the direction of the effect depending on the initial level of receptivity (Thody Wilson & Everard, 1979, 1981; Wilson, Thody & Everard, 1979). In females primed with estrogen alone, MSH facilitated lordosis in non-receptive females but did not inhibit receptivity in receptive females. Intracerebroventricular infusions of MSH or $ACTH^{4-10}$ also facilitate but do not inhibit lordosis in EB plus P primed females.

These studies are plagued with methodological problems, not the least of which is the fact that MSH injection/infusion occurs 4 to 6 hours before behavioral testing. With such long latency to drug effects, the potential for indirect (e.g., adrenal) mechanisms for the activation of lordosis increases. Animals were also compared for lordosis responsiveness in response to priming (plus drugs) across successive weeks, without counterbalancing the drug order and without demonstrating that responses were consistent from week to week. Finally, the division of animals into receptive and non-receptive groups for analysis helps statistically, but it obscures the large variability in response to the priming regimen used in control tests. This highly variable baseline may confound or "produce" drug effects. Thus, while MSH has been reported to influence lordosis behavior, the dose-dependency and "rate-dependency" of the drug effect make this proposal less than compelling.

Adrenocorticotrophic hormone (ACTH) stimulates sexual receptivity in estrogen-primed female rats when administered systemically (de Catanzaro & Gorzalka, 1980; Feder & Ruf, 1969), but these authors have pointed out the potential for an indirect mechanism involving activation of adrenal steroidogenesis and steroid secretion by ACTH. This criticism is supported by the study of de Catanzaro, Gray & Gorzalka (1981), in which OVX and OVX-adrenalectomized estrogen primed female rats were injected systemically with $ACTH^{1-24}$. Adrenalectomy prevented the facilitation of lordosis observed 120 minutes after $ACTH^{1-24}$ injection. No facilitation occurred in a test 20 minutes after $ACTH^{1-24}$ injection. Systemic $ACTH^{1-24}$ therefore induces sexual receptivity by a long-latency, adrenal-dependent mechanism which is thought to rely on adrenal secretion of progestins. The proposal of a neurotransmitter or neuromodulator role for ACTH is not necessary to explain the facilitation of lordosis following systemic administration.

In contrast to the peripheral effects of systemically administered $ACTH^{1-24}$, intracerebroventricular infusion of $ACTH^{1-24}$ inhibited receptivity in estrogen-primed OVX and adrenalectomized-OVX rats. This short-latency (20 minutes), central effect was independent of the adrenal glands. Thus, it appears that central ACTH can suppress lordosis behavior (de Cantanzaro et al., 1981).

Beta-endorphin, another pituitary peptide from the proopiomelanocortin precursor molecule, also influences lordosis behavior. Like ACTH, beta-endorphin has been found to reduce receptivity following intracerebral infusion; effective sites are the third ventricle (Weisner & Moss, 1984) and the MCG (Sirinathsinghji, Wittington, Audsley & Fraser, 1983; Sirinathsinghji, 1984). In support of an inhibitory role of endogenous opiates, intracerebral naloxone infusion was found to facilitate lordosis in one of these studies (Sirinathsinghji et al., 1983). While one study indicates that systemic administration of an opiate antagonist will facilitate lordosis (Allen, Renner & Luine, 1985) another study did not confirm this (Weisner & Moss, 1984).

In summary, a variety of peptides have recently been shown to influence lordosis behavior. Evidence is quite consistent in indicating a facilitative role of LHRH, which is particularly interesting since extrahypothalamic LHRH content appears to be modulated by estrogen (Shivers, Harlan, Morell & Pfaff, 1983). It is therefore possible that LHRH is an endogenous transmitter involved in the expression of hormone-dependent feminine sexual behavior. Oxytocin also facilitates lordosis, while vasopressin inhibits it. Systemic administration of alpha-MSH or ACTH can facilitate lordosis, but intracerebral ACTH suppresses receptivity. Beta endorphin also suppresses receptivity,

and data using opiate antagonists indicate that endogenous opiates may normally inhibit lordosis behavior.

Acetylcholinergic neurotransmission

The influence of acetylcholine on lordosis behavior is currently the main focus of several workers in the Hormones and Behavior Laboratory at Michigan State University; one aspect of cholinergic function in the regulation of sexual receptivity is the topic of this thesis. In order to provide background material for the discussion that will follow, it is necessary to include a more detailed description of cholinergic transmission than was provided for the other transmitters that have been discussed.

Acetylcholine was the first neurotransmitter to be identified, and it was among the first to be characterized pharmacologically (see McGeer, Eccles & McGeer, chapters 3 and 5 for review). Early work on the autonomic nervous system identified a set of effects which could be attributed to the action of acetylcholine. Some of these effects of acetylcholine were found to be mimicked by the alkaloid muscarine, while other actions were mimicked by nicotine. Some drugs, such as the belladonna alkaloids (atropinics) were found to selectively block the "muscarinic" effects. Other drugs blocked "nicotinic" effects of acetylcholine and nicotine. As the pharmacology of acetylcholine developed, so did the concept of two types of receptors, muscarinic and

nicotinic, which mediated separate subsets of the effects of acetylcholine.

More recent work has extended the muscarinic-nicotinic dichotomy to the central nervous system. Some behavioral effects of acetylcholine have been analyzed pharmacologically, and it has been possible to attribute some effects to muscarinic receptors, while other effects seem to be due to nicotinic receptors (e.g., Buccafusco & Brezenoff, 1980; Schechter & Rosecrans, 1971). Direct evidence for separate populations of nicotinic and muscarinic receptors in nervous tissue has been provided by receptor assays. Muscarinic receptors are selectively labeled by radioactive muscarinic antagonists (e.g., atropine, scopolamine, quinuclidinyl benzilate, N-methyl 4-piperidyl benzilate) and agonists (cis methyldioxolane)(see Kloog & Sokolovsky, 1978; Birdsall & Hulme, 1976 for citations). The addition of unlabeled nicotine or mecamylamine (a nicotinic antagonist) to the incubation medium does not reduce binding of the labeled muscarinic ligand, indicating that muscarinic receptors do not bind nicotinic drugs (Ehlert, Dumont, Roeske & Yamamura, 1980; Kloog & Sokolovsky, 1978; Yamamura & Snyder, 1974). In contrast, unlabeled muscarinic agents reduce binding of the radioligand, indicating that muscarinic drugs compete with the labeled ligands for muscarinic binding sites. Similarly, specific nicotinic receptors have been identified in binding studies with radiolabeled acetylcholine in the

presence of unlabeled atropine or with labeled nicotine itself (Clarke, Schwartz, Paul, Pert & Pert, 1984; Rainbow, Schwartz, Parsons & Kellar, 1984; Romano & Goldstein, 1980; Schwartz, McGee & Kellar, 1982). Results of competitive binding studies indicate that these sites are selective, and do not bind muscarinic agents with high affinity (Schwartz et al., 1982; Romano & Goldstein, 1980). One exception to this general rule is carbachol, which may be like acetylcholine in binding both muscarinic and nicotinic receptors (Schwartz et al., 1982; Romano & Goldstein, 1980). Many early studies used alpha-bungarotoxin as a nicotinic receptor ligand, but recent evidence has indicated that alpha-bungarotoxin may not bind to the same population of sites as nicotine and acetylcholine (e.g., Clarke et al., 1984).

Psychopharmacology of acetylcholine (ACh)

The first studies analyzing the influence of the cholinergic system on sexual receptivity in rats used systemic drug treatments and were performed by Lindstrom (1970, 1971, 1973, 1975; and Linstrom & Meyerson, 1967). Initial studies demonstrated an early (10 to 30 minute latency) inhibitory effect on lordosis after injection of muscarinic cholinergic agonists, which was blocked by the muscarinic antagonist atropine. The inhibition of lordosis by cholinergic agonists was potentiated by pretreatment with

monoamine oxidase inhibitors (pargyline, nialamide, or desmethylimipramine), and it was blocked by pretreatment with monoamine depletors (pCPA, reserpine, or tetrabenazine). These latter results suggested an interaction with monoaminergic systems; serotonin was thought to be involved because pretreatment with pCPA blocked the pilocarpine-induced inhibition of receptivity, but the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine did not. Considering the confusion regarding the mechanism of action of pCPA, this conclusion seems premature.

Facilitation of lordosis by systemic treatment with cholinergic agonists has also been reported (Lindstrom, 1975). The muscarinic agonist oxotremorine was found to increase lordosis behavior in EB primed OVX rats, and the facilitation was blocked by atropine. This cholinergic facilitation of lordosis was eliminated by adrenalectomy or hypophsectomy (Lindstrom, 1973), however, indicating that it may be mediated by adrenal secretions. Consistent with a facilitative role of muscarinic cholinergic transmission, Singer (1968) demonstrated that atropine suppresses lordosis in hormone-primed female rats.

Thus, from these systemic studies it appeared that muscarinic cholinergic drugs could either facilitate or inhibit lordosis, and muscarinic antagonists inhibited receptivity.

The contribution of the nicotinic receptor in the

regulation of sexual receptivity was first assessed by Fuxe, Everitt & Hokfelt (1977). These authors demonstrated that systemic injection of nicotine facilitated lordosis behavior in EB-primed OVX rats 5 minutes after injection. Pretreatment with the nicotinic receptor antagonist, mecamylamine, completely prevented the nicotine-induced facilitation of lordosis. In the remainder of this study and in a series of studies which followed, Fuxe et al. were more interested in the possible interactions of nicotine with dopaminergic systems than in the cholinergic system. As a result, they did not pursue the potential influence of other nicotinic cholinergic drugs on sexual receptivity.

A series of studies from this laboratory have demonstrated that intracerebral pharmacological stimulation of cholinergic receptors stimulates sexual receptivity in estrogen-primed OVX rats. This short-latency, transient effect is estrogen-dependent, progesterone independent, and independent of the adrenal glands.

Estrogen-dependency has been demonstrated by showing that cholinergic agonists are more effective in facilitating sexual receptivity after estrogen priming, even with very low doses of estrogen. Progesterone-independence has been demonstrated by showing that the cholinergic facilitation of lordosis occurs at levels of estrogen priming which are ineffective in facilitating lordosis in combination with progesterone (Clemens et al., 1981). Only a single

progesterone dose was used in this study, however. Further evidence for progesterone-independence comes from studies demonstrating that cholinergic agents can facilitate lordosis behavior in estrogen-primed animals which are behaviorally insensitive to progesterone. Specifically, males (Weaver, 1982; Weaver & Clemens, 1983) and females made unresponsive to progesterone-induced facilitation of lordosis either by a progesterone antagonist (Richmond & Clemens, 1985b) or by sequential or concurrent inhibition paradigms (Barr, Meyers & Clemens, 1984) nevertheless respond to intracerebral infusion of a cholinergic agonist.

The cholinergic agonists which have been used to facilitate lordosis in estrogen-primed female rats include carbachol, bethanechol, oxotremorine and pilocarpine (Clemens et al., 1983). While these drugs are primarily muscarinic in their actions, carbachol and bethanechol may stimulate nicotinic receptors (either directly or through stimulating release of endogenous ACh). The acetylcholinesterase inhibitor eserine (physostigmine) also facilitates lordosis following intracerebral infusion to estrogen-primed OVX rats, either when infused alone or in combination with ACh (Clemens et al., 1983).

In addition to the facilitative effect of pharmacological stimulation of the cholinergic system on lordosis, other studies have demonstrated that muscarinic transmission is required for hormone-induced sexual receptivity. Intracerebral infusion of a choline uptake

inhibitor, hemicholinium-3, which reduces endogenous ACh formation, disrupts receptivity in EB plus progesterone primed females (Dohanich & Clemens, 1980). Furthermore, infusion or application of muscarinic cholinergic antagonists (atropine and scopolamine) also disrupts EB plus P induced sexual receptivity (Clemens et al., 1983; Dohanich & Clemens, 1980; Kaufman, Pfaff & McEwen, 1984). These studies have indicated that there is activation of the cholinergic system in hormone-induced receptivity, and disruption of cholinergic transmission will disrupt receptivity.

Biochemical analysis of sites known to be important for sexual behavior has shown that cholinergic enzymes and receptor levels can be altered by hormone treatments. Whether these hormone-induced alterations in cholinergic neurochemistry are causally related to the induction of sexual receptivity is unclear, however. Thus, while estrogen treatment increases muscarinic cholinergic receptor number in the mediobasal hypothalamus of OVX female rats (Rainbow et al., Dohanich et al., 1982; Meyers & Clemens, 1985), the functional significance of this increase is unclear. Male rats, which respond with lordosis behavior in a manner not different from females to both estradiol treatment and estradiol plus cholinergic infusion (Weaver & Clemens, 1983), do not show alterations in hypothalamic muscarinic receptors following estrogen treatment (Dohanich et al., 1982). In addition, doses of estrogen which do

not significantly alter muscarinic receptors in female rat hypothalamus are effective in potentiating the response to infusion of muscarinic cholinergic drugs (Meyers & Clemens, 1985). In the absence of conclusive evidence that cholinergic drugs act in the mediobasal hypothalamus, the relevance of MBH muscarinic receptors to sexual receptivity is unclear. Similar criticisms can be made for other proposed sites.

Similarly, hormone-induced site-specific alterations in cholinergic enzymes have been demonstrated. It is possible that steroid hormones induce sexual receptivity by enhancing cholinergic transmission through suppressing acetylcholine degradation or enhancing acetylcholine synthesis. The activities of choline acetyltransferase, (the enzyme for ACh synthesis), and acetylcholinesterase (the enzyme which breaks down ACh) in hypothalamus are altered by estrogen treatment (Luine & McEwen, 1983; Luine & Rhodes, 1983; Luine, Khylchevskaya & McEwen, 1974, 1975). Meyers and Clemens (personal communication) have similarly demonstrated that estrogen treatment resulted in appropriate changes in cholinesterase activity in cerebrospinal fluid, hypothalamus, and MCG. The necessity of these neurochemical changes for the induction of receptivity are unclear, however. While these sites have been implicated as important for the regulation of feminine sexual behavior by other methods, these sites are not particularly high in cholinergic markers and therefore may not be the sites

important for the cholinergic facilitation of lordosis. On the other hand, lesion studies have demonstrated that the MCG is an important site in the mediation of the cholinergic facilitation of lordosis (Richmond & Clemens, 1985a).

Statement of Purpose

As reviewed above, a great deal of evidence indicates that cholinergic transmission is important for the regulation of sexual receptivity in female rats. Considering the existence of two types of receptors for acetylcholine, it is reasonable to ask whether this effect is mediated by muscarinic or nicotinic receptors, or both. While it appears that the entire facilitative effect of cholinergic agents on lordosis can be blocked by the muscarinic antagonists, atropine and scopolamine, the facilitation of lordosis by nicotine injection raises the possibility that nicotinic transmission is also important. The six experiments reported in this thesis have been performed to more precisely characterize the nicotinic contribution to the regulation of sexual receptivity in female rats. To accomplish this, comparison of the behavioral effects of nicotinic and muscarinic agonists and antagonists and their interactions have been assessed.

GENERAL METHODS

Subjects

Female Sherman strain rats (Camm Research Industries, Inc., Wayne, NJ) were used in all experiments reported here. Animals were received when 60-70 days of age (190-215 grams) and allowed to acclimate to the laboratory for at least one week before any manipulations were performed.

Housing and animal care conditions

Animals were housed in pairs in stainless steel hanging cages (30 x 23.8 x 23.8 cm) from the time they were received; animals that subsequently underwent stereotaxic surgery (Experiments 4 and 6) were singly housed at the time of stereotaxic surgery. The animals in Experiment 1 were single housed after the third drug test (Week 3) and remained singly housed throughout the remainder of the experiment. Animals in the other systemic drug studies (Experiments 2, 3, and 5) were housed in pairs throughout the experiment.

The colony room was maintained on a 14:10 reverse light-dark cycle, with lights off at 11:00 local time (EST or EDT). Temperature was controlled automatically at 70 degrees Farenheit. Red and dim white lights were used by experimenters in the colony room during the dark phase of the light cycle.

Food (Tek-lad mouse/rat diet, Winfield IA) and tap water

were available at all times. The animals for Experiment 1 were given tetracycline (Professional Veterinary Laboratories, Minneapolis, MN; 1/2 teaspoon per 250 cc distilled water) in place of drinking water from the time of arrival until 3-5 days after ovariectomy (e.g., a total of 13 days).

Ovariectomy

Seven to ten days after arriving in the laboratory, all females were ovariectomized under Ketamine anesthesia (Vetalar, Parke-Davis Co., Morris Plains, NJ). Ovariectomies were performed using bilateral flank incisions; the stump of the ovarian vasculature was ligated with silk (Champion number 0), the muscle of the body wall sutured with gut (Ethicon 4-0), and the skin over the incisions was closed with wound clips (Justrite, Clay Adams, Parsippany, NJ).

Ovariectomy removes the primary source of endogenous hormones which affect sexual behavior (estrogen and progesterone) and therefore allows the experimenter control over the hormonal status of the animals.

Screening Pretest

Following ovariectomy, all females were screened to assess for normal responsiveness to exogenous gonadal hormones. This screening pretest occurred 7-12 days after

ovariectomy and was preceded by hormonal priming. Each female was injected with estradiol benzoate (EB, 0.5 μg/ animal/ day, i.m.) 72, 48 and 24 hours before behavioral testing and also received a single injection of progesterone (P, 0.5 mg/ animal, i.m.) 4 to 6 hours before behavioral testing. This sequential treatment with estrogen and progesterone mimics the sequence of hormone secretion from the ovaries during the estrous cycle (Butcher et al., 1974) and normally induces high levels of sexual receptivity in all females. This laboratory has used the criterion that an animal must achieve a lordosis quotient (see Behavioral Testing section) of 70 or more in order to be considered normally responsive to exogenous hormones; animals which failed to meet this criterion were excluded from subsequent experimentation. Of the 151 females screened for use in the 6 experiments reported here, 1 was excluded because of this criterion.

Behavioral testing

Behavioral tests for sexual receptivity were administered at varying times before and after experimental treatments. In all cases, behavioral tests were conducted under dim red illumination in small rooms adjacent to the main colony room. Tests were conducted during the first half of the dark phase of the light cycle, but never less than 1 hour after lights-out (e.g., normally 12:30 - 4:00 pm).

Each behavioral test consisted of scoring the response of the test female to each of 11 mounts by a sexually vigorous Long-Evans strain "stimulus" male. The stimulus male was allowed to adapt to the testing arena (45 x 50 x 58 cm, Plexiglas, with Sanicel bedding) for at least 5 minutes before introduction of the test female. In the event that a stimulus male became sexually inactive prior to completion of a behavioral test, the test female switched to another testing arena with a different male. The response of the female to each mount by the male was scored on the intensity scale described by Hardy & DeBold (1971) and responses scored as 1, 2, or 3 were considered lordosis. A lordosis quotient was calculated for each female after exclusion of the response to the first mount, so that LQ = # lordoses/ # mounts x 100, with the number of mounts always being 10.

Statistical analysis

Lordosis quotients are scores on a scale of 0 to 100, with the score of an individual animal actually being a multiple of 10 between 0 and 100. Because of the interval nature and non-normal distribution of these data, parametric statistics are not appropriate. All data have therefore been analyzed using non-parametric statistical procedures as described by Siegel (1956). To compare related groups (e.g., individuals over several time points) the Friedman two-way analysis of variance, Wilcoxon matched-pairs signed-ranks test, and sign test were used. For unrelated

groups, Kruskall-Wallis analyses of variance, Mann-Whitney U tests, and Chi-square tests were used. The abbreviation "ANOVA" will be used for "analysis of variance". The criterion for statistical significance was set at $p < .05$; tests were two-tailed unless othrwize noted.

Hormone and drug solutions

Estradiol-17Beta-3-benzoate (EB, Sigma Chemical Co., St. Louis, MO) and progesterone (P, Sigma) were dissolved in sesame oil (Sigma) and administered by intramuscular injection into the thigh. Injection volume for hormones (EB and P) was 0.10 cc per animal except in Experiment 1, where the EB doses were administered on a µg/kg basis by adjusting the volume of injection at the rate of .4 cc / kg.

Several drugs were injected intraperitoneally in saline; control injections of the saline vehicle alone were also made. In order to accommodate for differences in body weight, systemic drug treatments were administered on a mg/kg basis. Drug solutions were made up to be administered at the rate of 0.8 cc/ kg, so that the "average" 250 gram rat would receive a 0.2 cc injection. The injection volume was adjusted for individual body weights measured earlier the same day. The following drugs for systemic injection were obtained from Sigma Chemical Company: nicotine (free base), mecamylamine hydrochloride, atropine sulfate, and hexamethonium bromide.

Intracerebral Studies

In Experiments 4 and 6, intracerebral microinfusions of drugs were performed. The following sections describe the methods for intracerebral cannula construction, implantation, infusion and histological verification.

Cannula construction

Cannulae assemblies were constructed from stainless steel tubing (Small Parts Inc., Miami Florida). Two 12 mm long "guide cannulae" (HTX-23 thin-walled) were fused with dental acrylic so that they were 2 mm apart and parallel to one another. These guide cannulae assemblies were permanently implanted into the brain as described below.

Removable "occluding inserts" were constructed from a short piece (3-5 mm) of HTX-23 gauge tubing crimped around one end of a longer piece (17-20 mm) of HTX-27 gauge tubing. A 13 mm shaft of HTX-27 tubing thus extended from the HTX-23 piece. When the HTX-27 portion of an occluding insert is inserted into a permanently implanted HTX-23 guide cannula, the HTX-23 gauge end of the insert only allows it to be lowered 13 mm into the guide. In this way, the occluding insert terminates one mm past the end of the guide cannula. When in place, the insert extends 1 mm past the end of the guide.

Infusion inserts were constructed of HTX-27 or HTX-28

gauge stainless steel tubing attached to 20 gauge tubing (PE-20; Clay Adams, Parsippany NJ) so that 13 mm of HTX tubing extended out of the PE tubing. In this way, the PE tubing would allow the HTX infusion insert to be inserted only 13 mm into the guide, e.g., so that the infusion insert terminated 1 mm past the end of the guide cannula in the same spot that the occluding insert had terminated.

Cannula implantation

Animals for some experiments underwent stereotaxic surgery for the implantation of intracerebroventricular (ICV) cannulae 1 to 12 days after the screening pretest. Surgery was performed under Ketamine anesthesia (approximately .14-.18 cc per animal). Following anesthetization, the head was shaved and the animal was placed in the stereotaxic instrument (David Kopf Instruments, New York). Using blunt ear bars and with the incisor bar at interaural zero, bilateral guide cannula assemblies (described above) were implanted. The assemblies were placed on a 27-gauge carrier and directed at the lateral ventricles by orienting the assembly over Bregma (A-P), and centering the two cannula over the sagittal suture (each was then 1 mm lateral to the sagittal suture). Holes were drilled in the skull with a dental drill, and the assembly was lowered 2.7- 2.8 mm from the level of dura. The guide cannula assembly was secured to the skull by placing dental acrylic over the base of the assembly and

over 4 small screws previously drilled into the skull. The dental acrylic was allowed to dry, the carrier assembly was removed, and the guide cannulae were fitted with removable occluding inserts. Finally, the skin over the skull was closed using metal wound clips. Following removal from the stereotaxic instrument, animals were allowed to recover from the anesthesia under observation before being placed individually into metal cages.

Intracerebral infusion technique

To perform an intracerebral infusion, the PE tubing attached to the infusion insert was first filled with the appropriate solution for infusion. The occluding inserts were removed, and the solution was delivered through the infusion insert into the lateral ventricle using a reciprocal microinfusion pump (Harvard Apparatus, Millis MA). The infusions were performed bilaterally, with each occluding insert being replaced immediately after removal of the infusion insert.

The volume of solution delivered was regulated not by measuring the amount of time at a given pump setting, but rather by watching the movement of a bubble drawn up into the infusion tubing. The bubble also served to separate the CSF and drug solutions. The bubble method was used because the rate of flow through an infusion insert can be altered by movement of the animal being infused, by movement of the infusion insert within the guide cannula, by the tissue

resistance, and by the height of the animal relative to the infusion pump (personal observations and personal communications from Drs. Gary Dohanich and Gail Richmond). On average, infusion of .5 microliters took 15-45 seconds at the pump settings used.

Infusion inserts (both the PE and HTX tubing portions) were used for a single drug only to avoid cross-contamination. Infusions were performed under dim white illumination in the colony room. The animals were gently restrained by holding them against the infuser's chest (lab coat required!); this method allows infusion of unanesthetized animals with minimal stress to either party.

Artificial cerebrospinal fluid vehicle

Mecamylamine (Experiment 6) and eserine (Experiment 4) (Sigma Chemical Co.) were dissolved in an artificial cerebrospinal fluid (CSF) vehicle immediately before intracerebroventricualr infusion; in Experiment 6 the CSF vehicle was also infused alone as a control. The CSF vehicle consisted of 130 mM NaCl, 25 mM $NaHCO_3$, 0.5 mM Na_2PO_4, 3.0 mM KCl, 0.8 mM $MgCl_2$, and 1.3 mM $CaCl_2$ in double distilled water, adjusted to pH 6.8. The CSF was allowed to warm to room temperature for approximately 1 hour prior to dissolving drugs in it.

Histological verification of cannulae location

Following completion of infusion studies, the animals

were anesthetized with pentobarbital (15 mg/ animal, i.p.) and perfused transcardially with 0.9% saline followed by 10% phosphate buffered formalin. Following perfusion, intact skulls were placed in 4% formalin for storage for several days; the brains were then removed from the skulls and returned to 4% formalin for several more days. The brains were removed from the formalin, rinsed in 100% ethanol, blocked, and frozen sectioned at 50 microns. The cortex on one side of the brain was often notched to aid in distinguishing right from left in the sections. Sections containing portions of the cannulae tracks were placed on slides previously coated with 3% gel and allowed to dry. Staining of the tissue and coverslipping was not performed, as these procedures were not necessary to determine whether the cannulae terminated in the ventricles. The sections were examined by projection and the site of termination of the cannulae was determined independently by two observers (the author and David A. Brigham). Only those animals for which both observers determined that both cannulae were in the ventricles have been included. The set of coordinates used placed the majority of cannulae in the lateral ventricles (bilaterally) at a level equivalent to Figure 17 in the atlas of Konig & Klippel (1963). The range of representative sections spans Figures 15 to 18 in Konig & Klippel. A section between Plates 14 and 15 in the atlas of Paxinos and Watson (1982) would also closely represent the typical section containing the cannulae tracks.

EXPERIMENT 1

As noted in the literature review portion of the INTRODUCTION section, Fuxe et al. (1977) have reported that systemic injection of nicotine facilitates lordosis behavior in estrogen-primed ovariectomized (OVX) rats. Experiments 1, 2, and 3 were performed to replicate those experiments (with slight modifications in the methods) and to extend the pharmacological analysis of nicotine action.

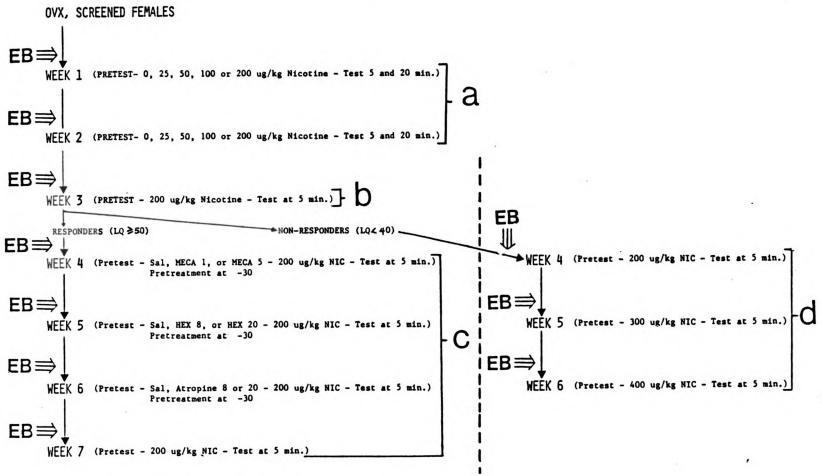
Procedure

Beginning one week after their screening pretest, 24 OVX Sherman strain rats were tested at one week intervals for lordosis behavior. Each weeks' testing consisted of a series of behavioral tests before and after pharmacological treatments. Each weekly test series was preceded by injections of EB (.5 µg/ kg) 72, 48, and 24 hours before behavioral testing. For clarity, the experiment will be discussed in four parts, referred to as Experiments 1a through 1d. Figure 1 is a schematic diagram of the procedure for Experiment 1.

Experiment 1a. For each of the first two weekly tests, the subjects were randomly assigned to one of five doses of nicotine (0, 25, 50, 100 and 200 µg/ kg). Tests for lordosis behavior were conducted before (pretest, PT) and 5 and 20 minutes after nicotine injection. The results of

FIGURE 1 Procedure, Experiment 1. Prior to each weekly series of lordosis behavior tests, the animals were primed with estradiol benzoate (EB, 0.5 µg/kg x 3). Experiments 1a, 1b, 1c, and 1d are indicated.

Experiment 1



these first two weeks have been pooled to provide a dose-response curve for nicotine on lordosis behavior in EB-primed OVX animals.

Experiment 1b. On the third week, all animals were EB-primed and given a single nicotine injection (200 µg/kg). Behavioral tests were conducted before (PT) and 5 minutes after nicotine injection.

For the subsequent weeks of testing, the animals were divided into two groups based on the results of these first three weeks of testing. It appeared that some animals were responding to nicotine injection, while other animals were not on these first tests. Groups of responders and non-respondes were therefore defined and they proceeded through the next several weeks on different schedules (Experiments 1c and 1d). Animals which had lordosis quotients of less than 40 on week 3 were designated as non-responders.

Experiment 1c. In order to determine whether animals that had been identified as non-responders were consistently non-responders to nicotine injection, they received nicotine injections of increasing dosage over weeks 4-6. Behavioral tests occurred before (PT) and 5 minutes after injection of nicotine. The nicotine dose was increased from 200 µg/kg (Week 4) to 300 µg/kg (Week 5) to 400 µg/kg (Week 6). Of the 9 animals designated as non-responders, 6 completed testing. The other three animals died before completion of the sixth test; these deaths did not seem to be related to

the drug treatments.

Experiment 1d. In order to obtain pilot data on which to base subsequent experimentation, the "responder" group (n = 15) underwent tests to determine the ability of several cholinergic antagonists to block the facilitation of lordosis by nicotine. Thus, "responders" were randomly assigned to one of three pre-treatment groups for each of the next three weeks. Each week, each animal was EB primed (.5 μg/ kg x 3), pretested for lordosis behavior, and pretreated with either saline, a low dose of antagonist, or a higher dose of antagonist. All animals subsequently received nicotine (200 μg/kg, 45 minutes after pretreatment), and lordosis behavior was assessed 5 minutes later. The nicotinic antagonists mecamylamine (1 and 5 mg/ kg, week 4) and hexamethonium hydrobromide (8 and 20 mg/kg, week 5), and the muscarinic antagonist, atropine sulfate (8 and 20 mg/kg, week 6) were used. A seventh behavioral test, which was identical in design to week 3, was also conducted. The EB-primed animals were simply tested for lordosis behavior before and 5 minutes after nicotine (200 μg/ kg) injection. The data from Experiments 1c and 1d should be considered preliminary data because of the low number of animals used and because the animals received several different drugs over time (Experiment 1d).

Results

Experiment 1a. The results of the first two weeks of

SYSTEMIC NICOTINE FACILITATED LORDOSIS

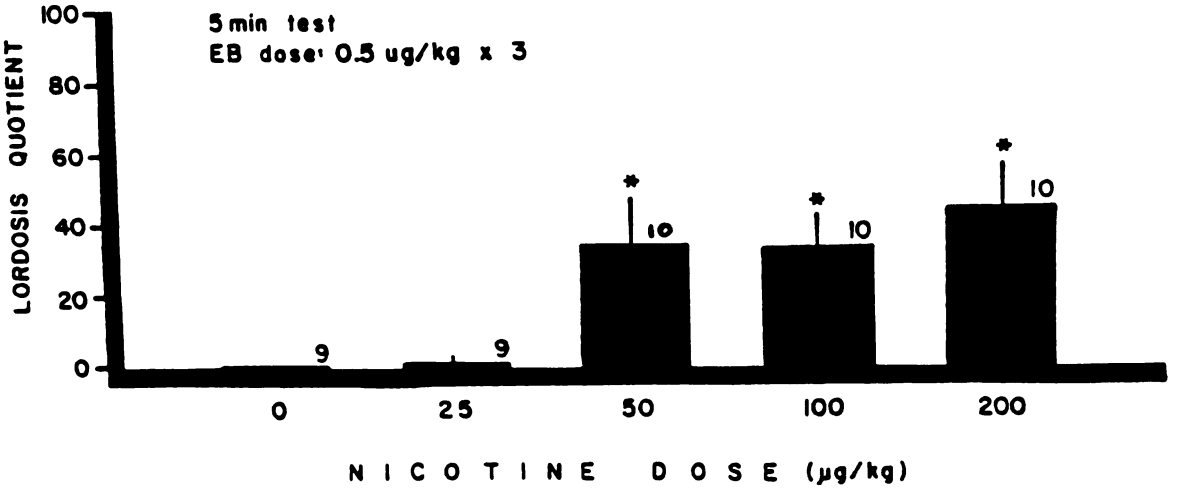


FIGURE 2 Mean lordosis quotients (+/- S.E.M.) of EB-primed OVX rats 5 minutes after injection of nicotine (0, 25, 50, 100 or 200 µg/kg, i.p.) (Experiment 1a). The stars indicate significant increases from pretest (PT) lordosis quotients (LQ's) for the 50, 100 and 200 µg/kg nicotine doses. Number of tests for each mean is 9-10 as indicated.

testing are presented in Appendix 1 and Figure 2. An overall effect of dose at the 5' test was demonstrated in that the proportion of animals showing lordosis behavior after treatment varied between nicotine doses (Chi-square test, X^2=17.9, 4 degrees of freedom, $p < .01$). Nicotine at doses of 50, 100 and 200 ug/kg produced a significant increase in lordosis quotients (LQ's) 5 minutes after injection (sign tests, PT vs 5 minute test [p=.031, .004, and .016, for doses 50, 100, and 200 µg/kg, respectively]). By 20 minutes after injection, LQ's had returned to low levels.

Experiment 1b. The response to a single nicotine dose (200 µg/kg) was quite variable. The average 5 minute test LQ was 49.5 (standard error = 6.6). The mean LQ for the responders (n = 15) was 72.1 +/- 4.2, while the non-responders (n = 9) averaged 16.7 +/- 4.4.

Experiment 1c. Table 1 shows the behavior of the non-responders to nicotine injection over weeks 3-6. Statistical analysis demonstrated that "non-responders" is a poor term for this group, as there actually was a significant facilitation of lordosis by nicotine on each week 3-5 (PT vs 5-minute LQ, p = .031, sign test). There was no effect of nicotine on week 6, possibly because this dose of nicotine (400 µg/kg) produced debilitation in some animals. It is clear from examination of Table 1 that animals selected as non-responders in week 3 are capable of showing induction of receptivity by nicotine injection. The

TABLE 1 Individual lordosis quotients of OVX, EB-primed "non-responders" before and 5 minutes after i.p. nicotine injection (Experiments 1b and 1c). Animals selected as nonresponders to 200 µg/kg NIC on week 3 received subsequent tests with 200, 300 and 400 µg/kg NIC.

NIC dose:	200 µg/kg Week 3		200 µg/kg Week 4		300 µg/kg Week 5		400 µg/kg Week 6	
#	PT	5'	PT	5'	PT	5'	PT	5'
162	0	20	10	60	0	50	10	30
163	0	30	10	70	0	70	0	30
169	0	0	0	0	0	60	0	10
174	0	20	0	100	0	60	10	0
181	0	10	0	10	10	10	0	0
184	0	10	0	30	0	0	0	0
MEAN	0	15.0	3.3	45.0	1.7	41.7	3.3	6.7
SEM	-	4.3	2.1	15.7	1.7	11.9	2.1	4.9

failure of animals to respond on week 3 was also apparently not due to insufficiency in dose, as most animals responded more strongly to the same dose of nicotine (200 μg/kg) on week 4.

Experiment 1d. The results of weeks 4-7 for the responding group of animals are presented in Appendix 2 and Figures 3-5. Nicotine injection resulted in a significant facilitation of lordosis in the saline pretreated animals each week (PT vs 5-minute test, sign test, $p = .031$ for each week 4-6).

In Week 4, there was a significant effect of pretreatment on lordosis behavior in the 5-minute tests (Kruskal-Wallis ANOVA; see Figure 3). Pretreatment with the tertiary nicotinic receptor antagonist mecamylamine (MECA, 1 or 5 mg/kg) completely blocked the response to nicotine (Mann-Whitney U-test, $p = .004$ for each MECA dose vs. saline; PT vs 5-minute test not significantly different, sign test).

Overall, hexamethonium (HEX) pretreatment did not significantly affect lordosis behavior in the 5-minute tests after nicotine injection (Kruskal-Wallis ANOVA, $p > .05$; see Figure 4). Two animals in the 20 mg/kg hexamethonium group had high pretest scores, however, and so it seems more appropriate to use the difference scores (5-minute test LQ - pretest LQ) for the analysis of the data from week 5. (For the other weeks, the difference scores and the 5-minute scored were very similar, and using either score produced

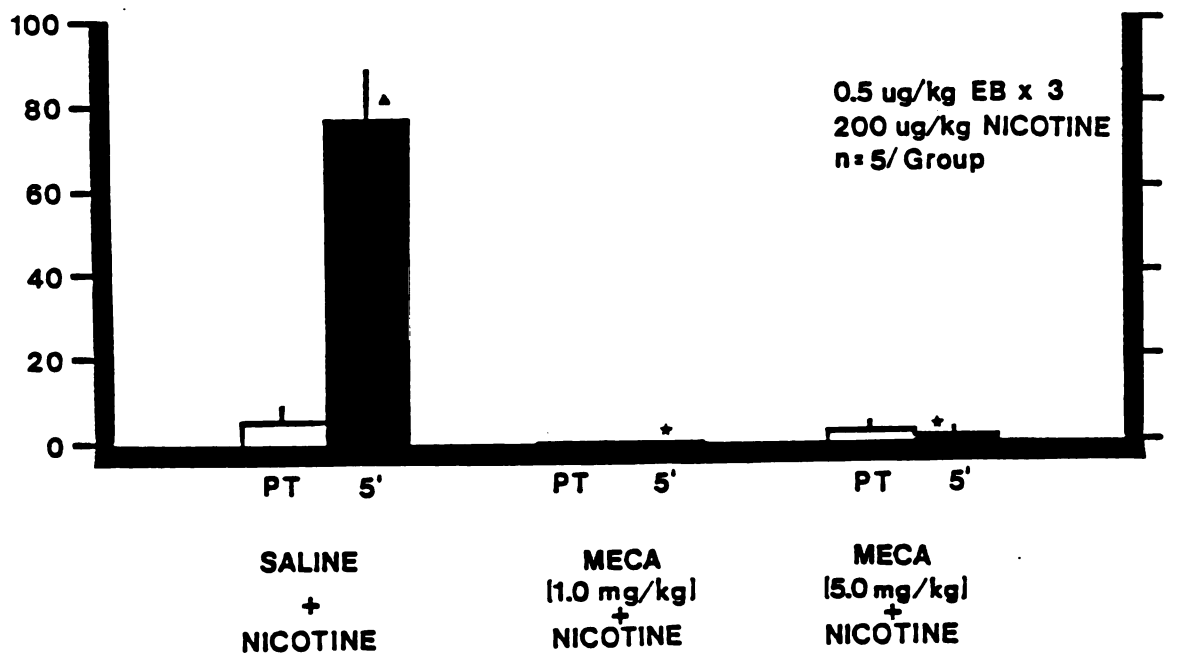


FIGURE 3 Mecamylamine (MECA) pretreatment prevented the facilitation of lordosis induced by nicotine (NIC) (Experiment 1d, week 4). The triangle indicates a significant increase from pretest LQ's. Stars indicate significant reductions in 5-minute LQ scores relative to the control group (saline + nicotine).

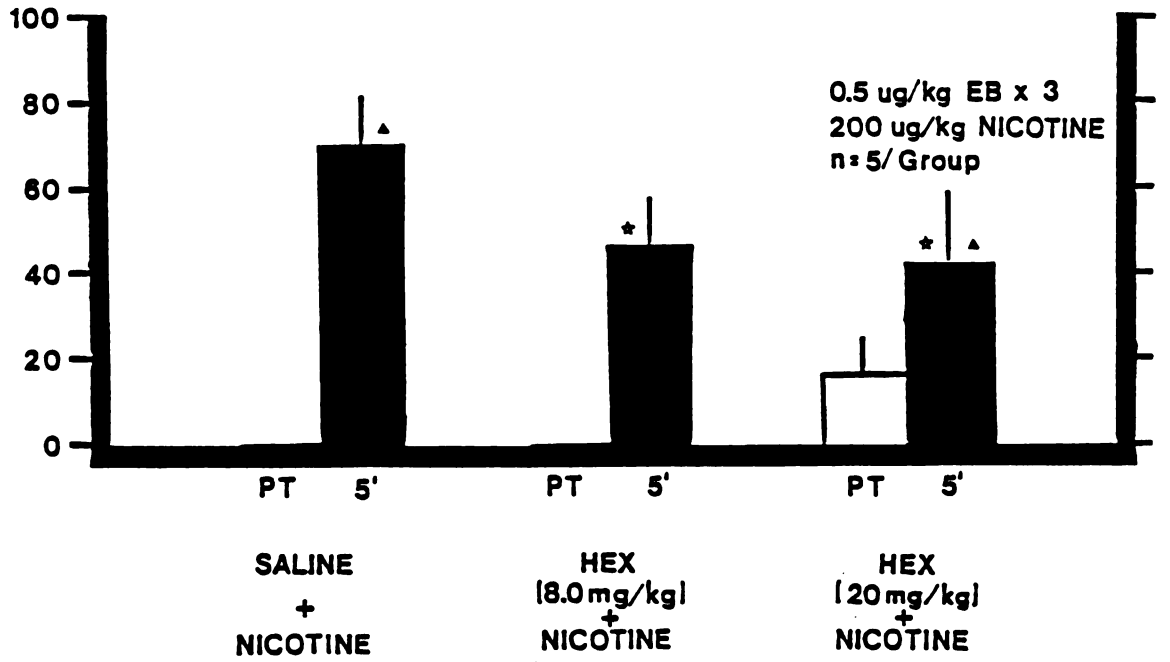


FIGURE 4 Hexamethonium (HEX) pretreatment reduced but did not prevent facilitation of lordosis by nicotine (Experiment 1d, week 5). The triangles indicates a significant increase from pretest LQ's. Hollow stars indicate significant reductions in LQ difference scores (5'-PT) relative to the control group (saline + nicotine).

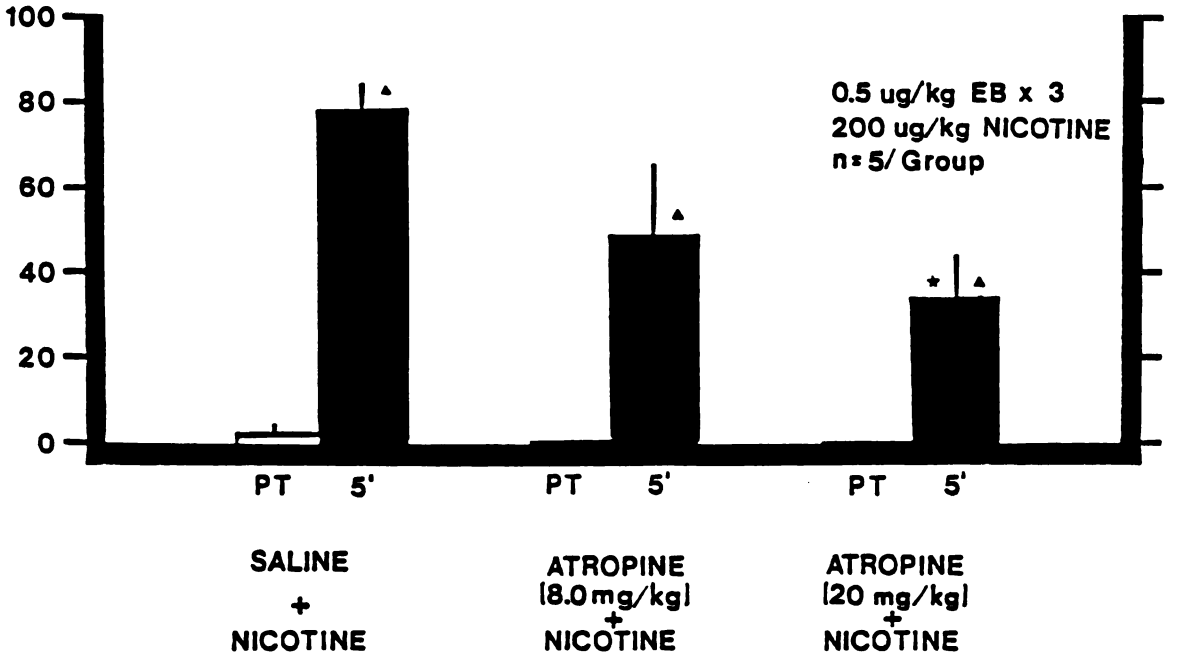


FIGURE 5 Atropine pretreatment reduced but did not prevent facilitation of lordosis by nicotine (Experiment 1d, week 6.) Triangles indicate a significant increase from pretest LQ's. Stars indicate significant reductions in 5-minute LQ scores relative to the control group (saline + nicotine).

the same statistical conclusion).

Analysis of difference scores revealed a significant effect of hexamethonium pretreatment (Kruskal-Wallis ANOVA, $p < .05$). Subsequent pairwize comparisons of the saline and hexamethonium pretreatment groups showed that hexamethonium pretreatment significantly reduced the facilitation of lordosis by nicotine as measured by the difference score (Mann-Whitney U-test, $p < .048$ for 8 mg/kg HEX and $p < .016$ for 20 mg/kg HEX vs saline pretreatment). Despite the reduction in nicotine-induced lordosis behavior by hexamethonium, there was still a significant facilitation of lordosis in the 20 mg/kg HEX group (sign test, $p = .031$) but not in the 8 mg/kg group.

While atropine pretreatment also significantly reduced lordosis behavior induced by nicotine injection (week 6), there was still a significant facilitation of lordosis in atropine-pretreated animals (see Figure 5; PT vs. 5-minute test, $p = .031$, for both atropine doses). Atropine (20 mg/kg) reduced 5-minute test LQ's relative to saline pretreatment (Mann-Whitney U test, $p < .008$); pretreatment with the lower atropine dose did not significantly affect the 5-minute test scores.

In week 7, the LQ of the responders averaged 49.3 (+/- 8.4) in response to 200 µg/kg nicotine.

Summary

The results of Experiment 1a demonstrate that nicotine

treatment can indeed facilitate lordosis in EB-primed female rats. The division of animals into responders and non-responders based on the response to a single injection of nicotine (Experiment 1b and 1c) was ineffective in identifying a responsive population of subjects, as the response of individual animals was not consistent over trials. The subsequent pharmacological analysis of nicotine responsiveness (Experiment 1d) indicates that the effect of nicotine on lordosis behavior is mediated by the nicotinic receptor, as the nicotine-induced facilitation of lordosis was completely blocked by the nicotinic antagonist mecamylamine. This agrees with the published results of Fuxe et al., (1977). Hexamethonium and atropine pretreatments also reduced the magnitude of the nicotine-induced lordosis, but a significant facilitative effect of nicotine was nevertheless seen in all but the 8 mg/kg hexamethonium group.

As noted earlier, the results from Experiment 1d were considered pilot data. In order to more fully examine the pharmacology of nicotine-induced lordosis behavior, the effects of pretreatment with mecamylamine and atropine were selected for further analysis in separate experiments, Experiments 2 and 3.

EXPERIMENT 2

Experiment 2 was designed to confirm the preliminary results of Experiment 1d, week 4. Specifically, the effect of pretreatment with the nicotinic antagonist mecamylamine on nicotine-induced receptivity was examined.

Procedure

Twelve Sherman strain female rats were OVX and screened as described in the GENERAL METHODS section. Three test series were administered at weekly intervals, with the first test series one week after the screening pretest. Each animal was EB-primed (0.13 µg EB at -72, -48, and -24 hours) before each weekly test series. For each test series, the animals were pretested for lordosis (PT), injected with mecamylamine (MECA, 2.5 or 10 mg/kg, i.p.) or the saline vehicle, and 30 minutes later injected with nicotine (150 µg/kg). They were then retested for lordosis behavior 5 minutes after nicotine. In this way, the ability of mecamylamine pretreatment to prevent the nicotine-induced facilitation of lordosis was assessed. Each animal received all three possible pretreatments over the three weeks of testing; the order of pretreatments was counterbalanced.

Results

As expected, nicotine injection (150 µg/kg) produced a statistically significant facilitation of lordosis in the

NICOTINE ANTAGONIST (MECAMYLAMINE) PREVENTED NICOTINE FACILITATION OF LORDOSIS

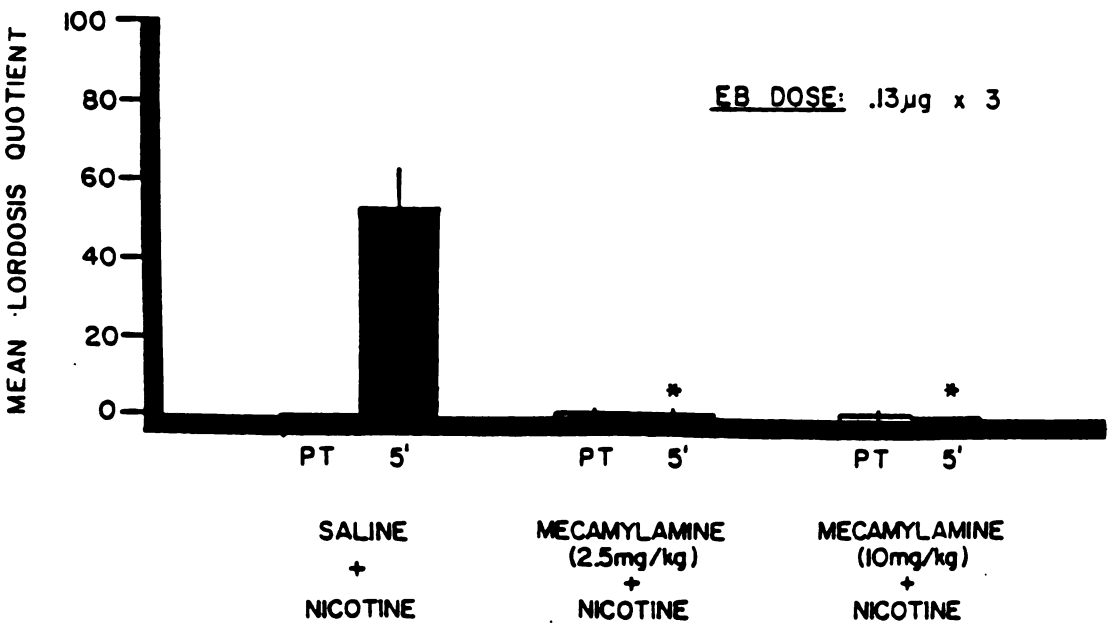


FIGURE 6 Mecamylamine pretreatment prevented nicotine facilitation of lordosis (Experiment 2). The triangle indicates a significant increase from pretest LQ's. Stars indicate significant reductions in 5-minute LQ scores relative to the control (saline + nicotine) test for these 12 animals.

Saline + Nicotine group (PT vs. 5-minute test, Wilcoxon matched-pairs signed-ranks test, $p < .05$). As illustrated in Figure 6, mecamylamine pretreatment (2.5 or 10 mg/kg) completely prevented the facilitation of lordosis by nicotine (150 µg/kg). This effect of pretreatment on the 5-minute test scores was statistically significant (Friedman two-way ANOVA, $p < .001$; followed by Wilcoxon matched-pairs signed-ranks test to compare saline vs each MECA week, one-tailed, $p < .005$). In none of the tests with mecamylamine-pretreatment did an animal show an increase in LQ following nicotine treatment, while these same animals all showed an increase in LQ in response to nicotine injection following the saline vehicle pretreatment.

Summary

In agreement with the preliminary results from Experiment 1 and with the published results of Fuxe et al. (1977), mecamylamine was found to prevent the nicotine-induced facilitation of lordosis. This indicates that nicotine is acting at nicotinic receptors to facilitate lordosis.

EXPERIMENT 3

Experiment 3 was performed to extend the pharmacological characterization of the nicotine-induced facilitation of lordosis. Specifically, Experiment 3 was designed to assess the ability of the muscarinic antagonist, atropine, to block the nicotine-induced facilitation of lordosis in EB-primed female rats.

Procedure

As in Experiment 2, OVX, screened Sherman strain rats (n = 10) were tested for lordosis behavior in 3 test series spaced one week apart. The first test series was one week after the screening pretest. Each week, each female was primed (.13 µg EB x 3), pretested for lordosis (PT), injected with a pretreatment, and 30 minutes later injected with nicotine (150 µg/kg, i.p.). Each animal was then retested for lordosis 5 minutes after the nicotine injection. The pretreatments were systemic injections of atropine (7.5 or 30 mg/kg) or the saline vehicle; each animal received all three pretreatments and the order of pretreatments was counterbalanced.

Results

The results of Experiment 3 are presented in Figure 7 and in greater detail in Appendix 3. Using within-animal comparison of the response over the three weeks (Friedman

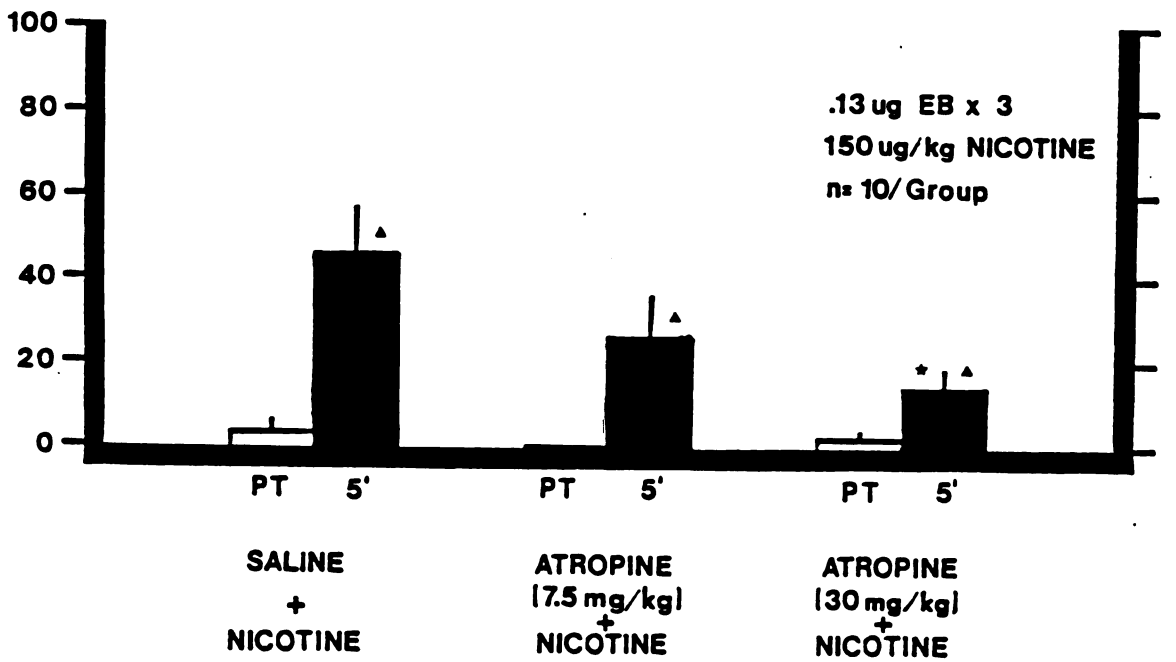


FIGURE 7 Atropine pretreatment reduced but did not prevent facilitation of lordosis by nicotine (Experiment 3). Triangles indicate a significant increase from pretest LQ's. The star indicates a significant reductions in 5-minute LQ scores relative to the control (saline + nicotine) test for this group of 10 animals.

two-way ANOVA), atropine pretreatment was found to be without effect on nicotine-induced lordosis behavior (calculated $X^2 = 4.55$ < critical value for significance, $X^2 = 5.99$ with two degrees of freedom, $p > .05$). Pre-planned comparisons between the results of the saline week versus each of the two atropine pretreatment weeks indicate that high dose atropine (30 mg/kg) pretreatment significantly reduces lordosis responding 5 minutes after nicotine relative to the saline-pretreated test (Wilcoxon matched-pairs signed-ranks test, T = 6.5 is less than 8, the critical value for 10 pairs at $p < .05$). Pretreatment with the lower dose of atropine (7.5 mg/kg) did not significantly reduce lordosis behavior in response to nicotine injection relative to the tests preceeded by saline pretreatment ($p > .05$, as the calculated T = 10 exceeds the critical value of T = 8 for 9 pairs).

Summary

Pretreatment with the higher dose of atropine (30 mg/kg) reduced the facilitation of lordosis behavior induced by systemic injection of nicotine (150 µg/ kg). The lower atropine dose (7.5 mg/ kg) was ineffective.

Atropine, a muscarinic antagonist, would not be expected to block an effect of nicotine mediated by the nicotinic receptor, as the nicotinic facilitation of lordosis is. The potential mechanisms for this effect of atropine will be considered in the GENERAL DISCUSSION section.

EXPERIMENT 4

Experiments 1, 2, and 3 examined the nicotine-induced facilitation of lordosis and its pharmacological basis. These studies clearly indicate that nicotinic receptor stimulation can facilitate lordosis. The work of others in this laboratory has indicated that intracerebral infusion of agents which stimulate muscarinic receptors will facilitate lordosis in EB-primed OVX rats, and infusion of agents that disrupt muscarinic cholinergic transmission disrupts ongoing EB plus P-induced sexual receptivity (see Psychopharmacology of Acetylcholine section of the INTRODUCTION. Some of the agents which have been used to stimulate muscarinic receptors also may result in stimulation of nicotinic receptors. The acetylcholinesterase inhibitor eserine (physostigmine) is an example. By blocking the enzymatic degradation of acetylcholine, eserine produces an increase in synaptic acetylcholine which could potentially stimulate both muscarinic and nicotinic receptors. The present experiment was designed to assess the contribution of nicotinic receptors to the eserine-induced facilitation of lordosis.

Procedure

A total of 51 Sherman strain rats were OVX, screened, and implanted with cannulae which were later determined to terminate bilaterally in the lateral ventricles. Two

lordosis test series were administered; the first test series was one week after stereotaxic implantation of the intracerebral cannulae, and the second test series was one week later. Prior to each weekly test series, each female was primed with EB (.13 μg x 3). Animals were pretested for lordosis behavior before any treatments (PT), injected with a pretreatment (mecamylamine or saline), and infused with eserine (5 μg/ 0.5 μl/ side, bilaterally) 30 minutes later. The animals were retested for lordosis behavior 15 minutes after ICV eserine infusion. Twenty-four animals received mecamylamine pretreatment at 5 mg/ kg, i.p., one week and the saline vehicle the other. Another group of animals (n = 27) received a higher dose of mecamylamine (10 mg/kg) one week and the saline vehicle the other. Each animal thus received two eserine infusions spaced one week apart; one week the eserine was preceded by one of the two doses of mecamylamine and the other week eserine was preceded by the saline vehicle. Order of the pretreatments was counterbalanced within each group of animals.

Results

Eserine infusion resulted in a significant facilitation of lordosis in control tests with animals receiving saline pretreatment (see Figure 8). Following mecamylamine pretreatment (either dose), eserine infusion still produced a statistically significant facilitation of lordosis (PT vs 15 minute test, Wilcoxon signed-ranks matched-pairs test,

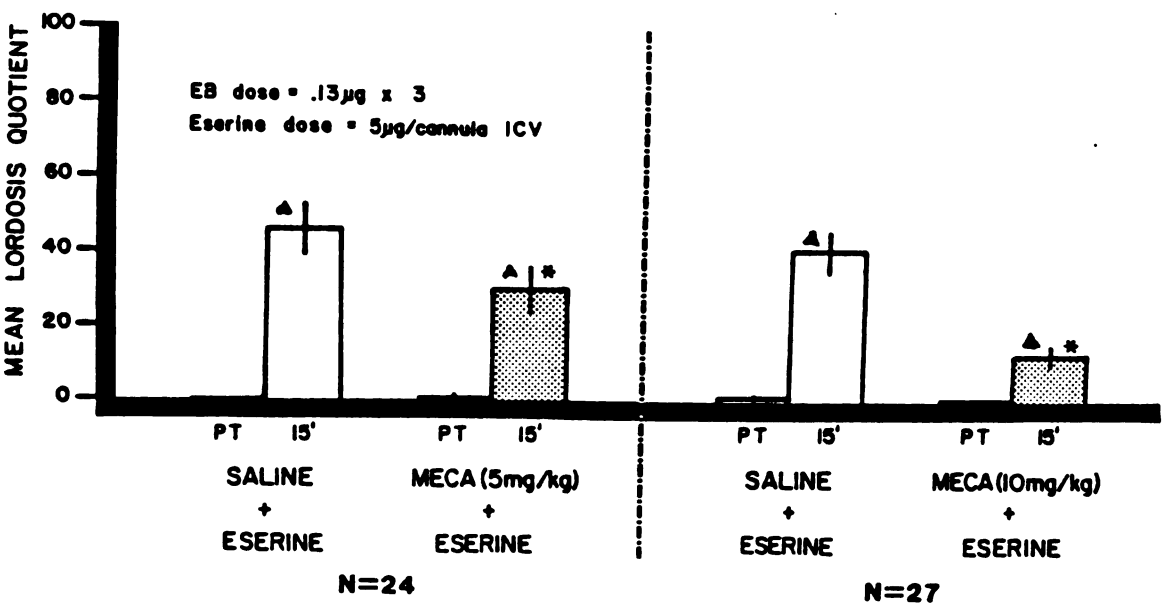


FIGURE 8 Mecamylamine pretreatment reduced but did not prevent the facilitation of lordosis induced by bilateral ICV eserine infusion (Experiment 4). Triangle indicate a significant increase from pretest LQ's. Stars indicate significant reductions in 15-minute LQ scores relative to the control (saline + eserine) test within each group.

$p < .05$). A comparison within animals across the two weeks, however, indicated that mecamylamine pretreatment reduced the eserine-induced facilitation of lordosis (15' tests compared within animal for saline vs MECA week, Wilcoxon matched-pairs test, $p < .05$). Thus, mecamylamine pretreatment reduced but did not prevent the induction of receptivity by ICV eserine treatment.

Summary

The finding that eserine facilitates lordosis behavior in EB-primed female rats agrees with previous results from this laboratory (Clemens et al., 1983). While mecamylamine pretreatment reduced the eserine-induced facilitation in this experiment, MECA did not completely prevent the facilitative effect of eserine.

EXPERIMENT 5

While the experiments described to this point have demonstrated that pharmacological stimulation of nicotinic receptors can influence lordosis behavior in rats, none of them has addressed the question of whether nicotinic receptor stimulatiion is necessary for sexual receptivity. Experiments 5 and 6 were designed to ask exactly that question, by assessing the effects of the nicotinic receptor blocker, mecamylamine, on hormone-induced sexual receptivity. In experiment 5, MECA was administered systemically, while in Experiment 6 it was infused ICV.

Procedure

Ten OVX, screened Sherman strain rats served as subjects. Three test series were administered one week apart, with the first test series occurring one week after the screening pretest. Prior to each weekly test series, each female was primed with EB (.5 µg x 3) plus P (.5 mg) in order to induce high levels of sexual receptivity on the day of testing. The animals were tested for lordosis behavior before (PT) and 15, 45 and 90 minutes after i.p. injection of MECA (5 or 10 mg/kg) or the saline vehicle. Each female received all three treatments, with treatment order counterbalanced.

SYSTEMIC TREATMENT WITH NICOTINE ANTAGONIST (MECAMYLAMINE) DID NOT INHIBIT LORDOSIS

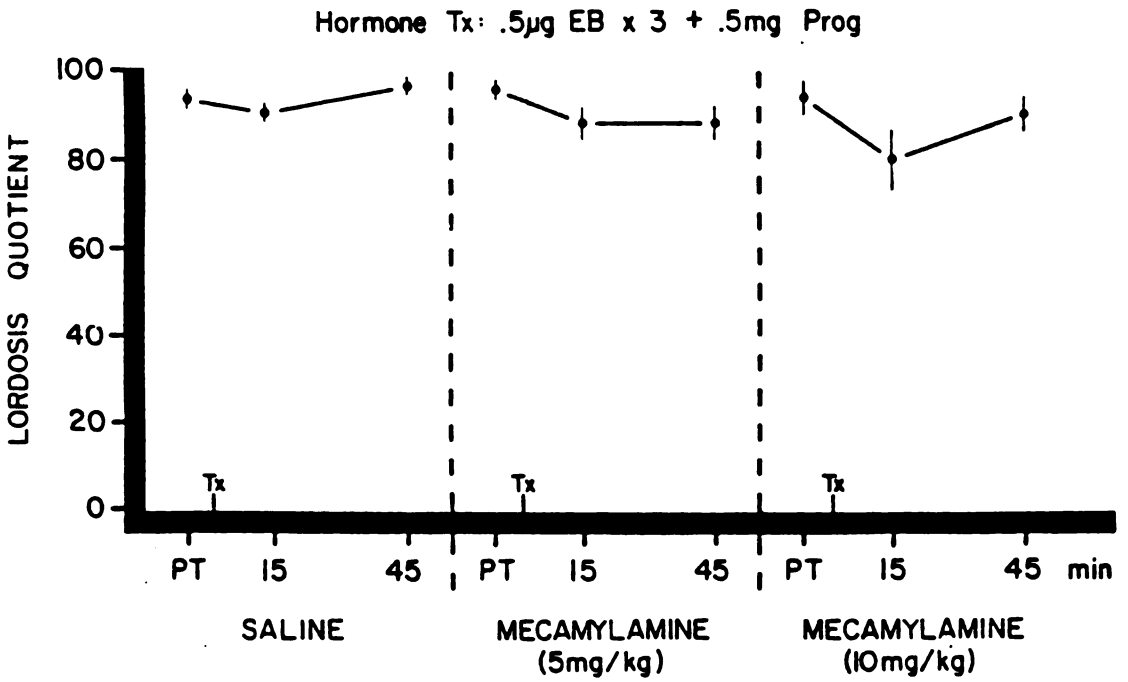


FIGURE 9 Systemic treatment (Tx) with mecamylamine (MECA) did not inhibit receptivity in EB plus P primed OVX rats. Ten animals received each of the three systemic treatments in three behavioral test series at weekly intervals. There was no effect of mecamylamine treatment at either dose.

Results

Systemic injection of the nicotinic receptor antagonist mecamylamine was ineffective in disrupting the hormone-induced receptivity (see Figure 9; Friedman two-way ANOVA of 15-minute test LQ's or delta-15 LQ's [= LQ_{15} - LQ_{PT}] across treatments within animals, $p > .05$). Only the 15-minute test scores and delta-15 scores were examined, as the peak "effect" was observed 15 minutes after injection.

Summary

Systemic mecamylamine treatment did not disrupt sexual receptivity in EB plus P primed females at doses which do block nicotine-induced lordosis and which reduce ICV eserine-induced lordosis behavior.

EXPERIMENT 6

In Experiment 6, the ability of ICV infusion of MECA to disrupt EB plus P-induced receptivity was assessed.

Procedure

Fourteen OVX, screened, Sherman strain rats with bilateral ICV cannulae (histologically verified after completion of the experiment) were used as subjects. Each rat underwent two testing sessions spaced one week apart, with the first of these occurring one week after the stereotaxic surgery for implantation of the intracerebral cannulae. Prior to each of the two testing sessions, each animal was primed with EB (0.5 µg/ day x 3) plus P (0.5 mg) in order to induce high levels of sexual receptivity on the day of behavioral testing. Animals were tested before (PT) and 15, 45 and 90 minutes after infusion. The animals were randomly divided into two groups (n=7 each), one of which received an infusion of a high dose of MECA (10 µg/ .5µl/ side, bilaterally) one week and the CSF vehicle the other week; the other group received a lower dose of MECA (5 µg/ .5µl/ side) one week and CSF the other week. Within each group, the order of infusion treatments was counterbalanced.

Results

Bilateral infusion of MECA did not significantly affect

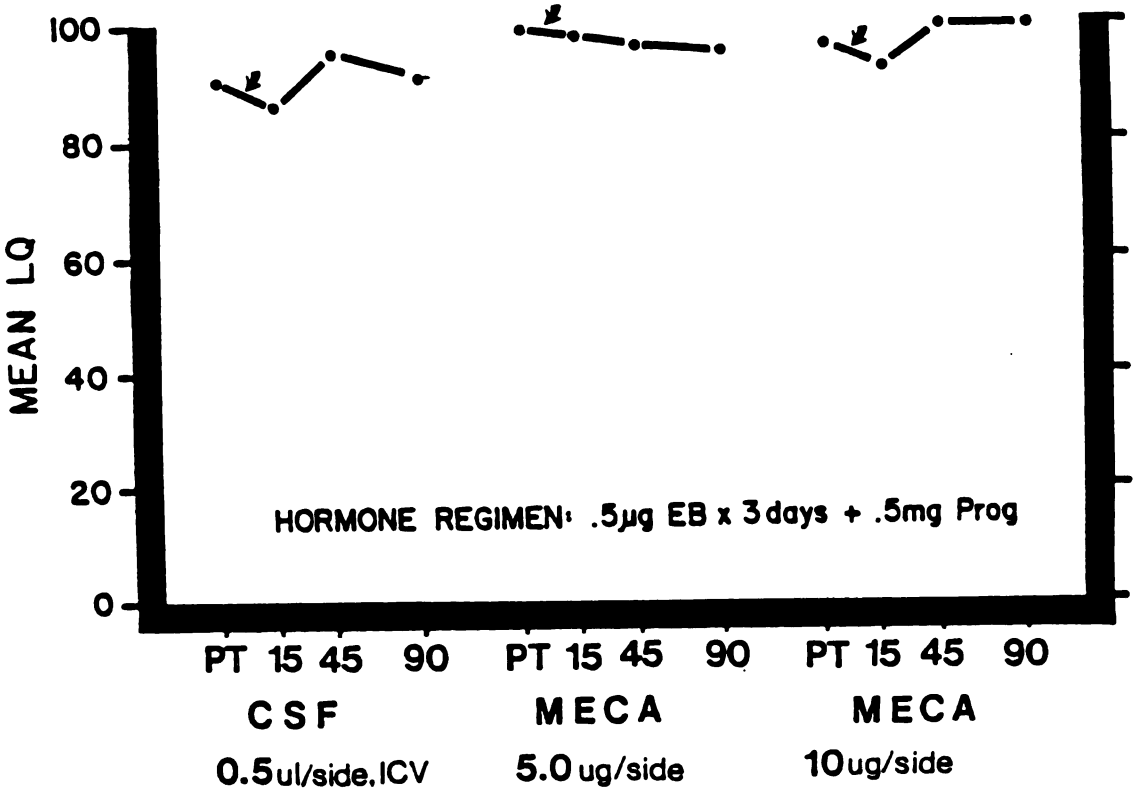


FIGURE 10. ICV infusion of mecamylamine did not inhibit receptivity in EB plus P primed OVX rats (Experiment 6). For each MECA dose, seven animals received MECA one week and the CSF vehicle the other week. The CSF tests from the two groups have been combined for clarity in this figure.

LQ's in EB plus P primed female rats (Wilcoxon matched-pairs signed-ranks test on 15 minute scores or delta-15 LQ's, comparing CSF and MECA weeks within animal, $p > .05$). The CSF tests from the two MECA dose groups have been pooled for clarity in Figure 10, which shows the response over time following infusion of mecamylamine and CSF.

Summary

Intracerebral infusion of mecamylamine was ineffective in antagonizing hormone-induced receptivity at the doses used.

GENERAL DISCUSSION

Experiment 1a demonstrated that nicotine treatment could facilitate lordosis in EB-primed female rats as reported previously (Fuxe et al., 1977). The nicotine-induced facilitation of lordosis was completely blocked by the nicotinic antagonist mecamylamine (Week 4 of Experiment 1, Experiment 2), also confirming the results of Fuxe et al.

Hexamethonium pretreatment reduced the magnitude of the nicotine-induced lordosis, but a significant facilitative effect of nicotine on lordosis was nevertheless seen in the 20 mg/kg HEX pretreatment group (Week 5 of Experiment 1d). Atropine pretreatment was also found to reduce nicotine-induced receptivity, but not to prevent it completely (Week 6 of Experiment 1d, and Experiment 3).

Systemically administered mecamylamine pretreatment reduced the induction of receptivity by ICV eserine infusion (Experiment 4). Systemic MECA was ineffective, however, in reducing receptivity in EB plus P primed females (Experiment 5). ICV infusion of MECA was also ineffective in disrupting hormone-induced lordosis behavior (Experiment 6).

The nicotine-induced facilitation of lordosis observed in Experiments 1, 2, and 3 agrees with the results of Fuxe et al. (1977) despite methodological differences. Specifically, these differences include the strain of animals used (Fuxe: Sprague-Dawley vs. our use of the Sherman strain) and the EB priming regimen (Fuxe: 1

µg/animal/day x at least 5 days, vs. our 0.5 µg /kg, or approximately 0.13 µg/animal/day x 3). The testing protocol also differed (Fuxe et al. did not pretest their animals before administration of nicotine) as did the form of nicotine (Fuxe: tartrate form, vs. our use of free base).

In estrogen-primed OVX rats, any drug can potentially facilitate lordosis by stimulating release of progesterone from the adrenal gland. There are two reasons that this potential hormonal mechanism is unlikely. First, the effect of nicotine is very rapid and transient, occurring within 5 minutes and declining by 20 minutes after injection. Direct intravenous injection of progesterone itself requires 30 minutes to facilitate lordosis (Glaser, Rubin & Barfield, 1983), so the effect of nicotine could not be mediated by progesterone. Second, the estrogen dose used in this study is below the level required for development of sensitivity to the behavioral effects of progesterone (Clemens et al., 1981).

The division of the population of animals used in Experiment 1 into responders and non-responders seemed necessary in attempting to screen the subject population. The response of animals to nicotine injection is not consistent over trials, however, making it impossible to assign an animal to one of these categories based on a single test. The ineffectiveness of the classification used is illustrated in Table 1. Selected non-responders did respond strongly to nicotine injection (Table 1), and

selected responders occasionally did not respond to nicotine (Experiment 1d, Week 7, individual data not shown). The variability between animals is apparently not due to consistent individual differences in susceptibility.

Pretreatment with the nicotinic antagonist mecamylamine completely blocked nicotine-induced lordosis behavior, while hexamethonium and atropine were less effective. This indicates that the behavioral effects of nicotine are due to an action on the nicotinic receptor.

The lower effectiveness of the nicotinic antagonist, hexamethonium, to block nicotine-induced facilitation of lordosis relative to mecamylamine may be due to structural differences between these two compounds. Both mecamylamine and hexamethonium are nicotinic antagonists, but hexamethonium has a quaternary nitrogen (and is therefore charged at physiological pH) while mecamylamine has an uncharged tertiary nitrogen. Due to its charge, hexamethonium would have poor access to the brain from the systemic circulation due to the blood-brain barrier (Goodman & Gilman, 1975). The partial effectiveness of hexamethonium to reduce nicotine-induced facilitation of lordosis may indicate that at least part of nicotine's effect is due to stimulation of peripheral nicotinic receptors. Alternatively, the brain areas in which nicotine acts to induce lordosis behavior may be poorly protected by the blood-brain barrier. Circumventricular organs and the hypothalamus would be indicated as important sites for

nicotine action by such a proposal. On the basis of the data on hand, it is impossible to distinguish between these alternatives.

The antagonism of nicotine by atropine, a muscarinic antagonist, was unexpected. There are, however, several possible explanations for this effect. If one assumes that nicotine facilitates lordosis only by binding to nicotinic receptors, then three possibilities exist. First, atropine may act as a nicotinic antagonist by competing with nicotine for nicotinic binding sites. Such a possibility is not supported by biochemical data, however, as atropine does not displace nicotine from nicotinic binding sites in vitro (see INTRODUCTION for citations). A second possibility is that atropine reduces nicotine-induced receptivity by preventing activation of muscarinic receptors by nicotine. In this scheme, it is proposed that nicotine's behavioral effect is in part dependent upon indirect activation of an endogenous muscarinic mechanism. Atropine reduces the effect of nicotine by acting "downstream" in the lordosis-facilitating neural circuit activated by nicotine. No assumption of interaction at a single set of receptors or within a single neural site is required. The potential for this type of interaction may be one of the reasons that the literature on the psychopharmacology of sexual behavior is so full of contradictory effects and neurotransmitter interactions. Finally, and most likely, atropine may antagonize the behavioral effect of nicotine in a non-specific manner. At

the high dose used (30 mg/kg), it is possible that atropine has non-specific effects not limited to the muscarinic receptor. The dose of atropine which reduced nicotine-induced receptivity is certainly higher than should have been necessary; the lower (7.5 mg/kg) dose is sufficient for most specific effects of atropine. This lower dose, while ineffective in antagonizing the nicotinic induction of receptivity, is sufficient to block the induction of receptivity by intracerebral infusion of muscarinic drugs (see Clemens et al., 1983 for review).

Other possible explanations for the antagonistic effects of atropine and nicotine on lordosis behavior involve assuming that nicotine does not act strictly at nicotinic receptors. Antagonism between atropine and nicotine would be expected if nicotine acted as a muscarinic agonist, either directly or indirectly. A direct agonist role of nicotine is unlikely, as nicotine is not a ligand for muscarinic receptors (see INTRODUCTION). Indirect roles are also possible. Nicotine could potentially release endogenous acetylcholine or to delay the breakdown of acetylcholine, although I am unaware of any biochemical data to support either suggestion. Either of these actions would be expected to facilitate lordosis behavior due to the resulting stimulation of muscarinic receptors. On the basis of other work in this laboratory, it appears that nicotine probably does not facilitate lordosis by an indirect action involving endogenous ACh. Atropine pretreatment at 2 mg /

animal, i.p. (approximately equal to the behaviorally ineffective lower dose used in Experiment 1d and Experiment 3) completely prevents the facilitation of lordosis induced by intracerebral infusion of carbachol, eserine, or acetylcholine plus eserine. These compounds stimulate lordosis behavior by stimulating muscarinic receptors directly or indirectly; if nicotine also stimulates lordosis by a muscarinic receptor mediated mechanism, then the lower dose of atropine should have completely prevented the facilitation of lordosis by nicotine.

On the basis of the discussion to this point, it is posible to conclude that the most likely mechanism of action of nicotine on lordosis behavior is through the stimulation of nicotinic receptors. The most likely mechanisms by which atropine could interfere with that facilitation is to block muscarinic receptors "downstream" from the site of nicotine binding or as a result of non-specific actions of the high dose used. It appears from other work in this laboratory that there is an important muscarinic cholinergic link in the neural circuit for lordosis behavior (see INTRODUCTION). Disruption of this atropine-sensitive link in the neural pathway leading to nicotine-activated lordosis behavior disrupts the behavioral response to nicotine. A partial behavioral response to nicotine persists, perhaps due to activation of other transmitter systems by nicotine.

The finding that ICV eserine facilitates lordosis behavior

in EB-primed female rats agrees with previous results from this laboratory (Clemens et al., 1983). While systemic mecamylamine treatment was found to reduce this facilitation in Experiment 4, it did not completely prevent it. This indicates that stimulation of muscarinic receptors can result in facilitation of lordosis, even if nicotinic receptors are blocked. As noted above, however, the converse is not true, as Clemens et al. (1983) have shown that atropine pretreatment completely prevents the facilitation of lordosis by eserine or eserine plus acetylcholine infusion. This pattern of results is not consistent with the nicotine data, however. While the behavioral effect of nicotine injection is blocked by mecamylamine and reduced by a high dose of atropine, the behavioral effect of eserine infusion is blocked by atropine and reduced by mecamylamine. The facilitation of lordosis by eserine is apparently mediated primarily by muscarinic receptors, although nicotinic receptor activation may contribute. As concluded earlier, the behavioral effect of nicotine is most likely due to activation of nicotinic receptors, which eventually results in the activation of an atropine-sensitive pathway. These results, when taken together, suggest that there is a common muscarinic mechanism to the facilitation of lordosis by both nicotine and eserine. It appears that nicotine may facilitate lordosis through pharmacological activation of the endogenous muscarinic mechanism, and that there is not

really a "nicotinic mechanism" for lordosis behavior.

This proposal is strongly supported by the results of Experiments 5 and 6. In these experiments, systemic or intracerebral treatment with mecamylamine did not disrupt sexual receptivity in EB plus P primed females. If there were an endogenous nicotinic mechanism relevant to hormone-activated sexual receptivity, then disruption of nicotinic transmission should have disrupted receptivity. This was not observed, even at doses which are behaviorally effective in other systems. The doses of mecamylamine administered systemically had earlier been found to prevent nicotine-induced lordosis and to reduce eserine-induced lordosis behavior (Experiments 1d, 2, and 4). While we have no behavioral data of our own regarding the doses of mecamylamine administered intracerebrally, Brezenoff & Jenden (1970) showed that 5 μg mecamylamine completely blocked a delayed pressor response produced by carbachol when both drugs were infused (sequentially) into the floor of the fourth ventricle. The doses of mecamylamine used therefore should have been effective in disrupting nicotinic transmission.

While mecamylamine is ineffective in disrupting lordosis behavior in hormone-primed animals, administration of a muscarinic antagonist in the same paradigms does interfere with sexual receptivity. Systemic treatment with the muscarinic antagonist scopolamine (doses) significantly reduces receptivity in EB plus P primed female rats

(Richmond & Clemens, 1985a). Similarly, ICV infusion of scopolamine reduces lordosis quotients in EB plus P primed female rats (Clemens et al., 1983). It appears that blockade of muscarinic receptors disrupts hormone-induced lordosis behavior, but blockade of nicotinic receptors does not. Once again, an endogenous muscarinic cholinergic mechanism is indicated.

Pharmacological stimulation of nicotinic receptors can facilitate lordosis, but stimulation of nicotinic receptors is not essential for hormone-induced sexual receptivity. In contrast, while pharmacological stimulation of central muscarinic receptors also induces sexual receptivity, stimulation of muscarinic receptors is critical for hormone-induced sexual receptivity. In conclusion, muscarinic cholinergic transmission is more important than nicotinic transmission in the regulation of lordosis behavior. In fact, we have no evidence to indicate that the behavioral effects of nicotine treatment are actually related to the control of lordosis behavior by endogenous nicotinic transmission. The behavioral effects of nicotine may represent a receptor-mediated pharmacological artifact, as these effects may have nothing to do with the regulation of sexual receptivity by a nicotinic mechanism. It therefore does not seem fruitful to attempt to determine the sites of nicotine action, as this effort may be one of studying the behavioral pharmacology of nicotine rather than studying the neurochemical control of sexual receptivity.

On the other hand, the study of the sites of the muscarinic mechanism is very important to the understanding of lordosis behavior. It is possible to propose that the muscarinic cholinergic mechanism being discussed represents the "final common pathway" to the expression of hormone-activated lordosis behavior. If this muscarinic mechanism is as important as it appears to be on the basis of the data currently available, then muscarinic antagonists should disrupt lordosis activated by any of a large number of hormonal or pharmacological methods. Only those agents which facilitate lordosis via activation of an alternate (non-muscarinic) route or via simultaneously activating parallel routes should facilitate lordosis in the presence of atropine or scopolamine.

An alternate suggestion is that pharmacological treatments which facilitate lordosis do so by presynaptic modulation of acetylcholine release. If this is the case, then muscarinic antagonists should prevent the behavioral effect of any of the agents which facilitate lordosis by increasing acetylcholine release. Conversely, muscarinic agonists should overcome the inhibition of lordosis induced by agents which are presumed to act by reducing acetylcholine release. The possibility that nicotine facilitates lordosis by stimulation of acetylcholine release has already been discussed, and it seems unlikely to this author.

SUMMARY AND CONCLUSIONS

Systemic nicotine injection facilitated lordosis behavior in estrogen-primed OVX rats, and this facilitation was prevented by pretreatment with the nicotinic antagonist, mecamylamine. Hexamethonium, a nicotinic antagonist with poor access to the brain, was less effective. Hexamethonium reduced but did not prevent the nicotine-induced facilitation of lordosis. Similarly, the muscarinic antagonist atropine reduced but did not prevent nicotine-induced receptivity.

ICV infusion of the cholinesterase inhibitor, eserine (physostigmine) facilitated lordosis; this effect was reduced but not prevented by mecamylamine pretreatment.

ICV infusion or systemic injection of mecamylamine did not reduce sexual receptivity in OVX rats made highly receptive with estrogen plus progesterone priming.

Previously published work from this laboratory has demonstrated that muscarinic antagonists (atropine and scopolamine) disrupt sexual receptivity when administered systemically or intracerebrally in the same paradigm (Clemens et al., 1980, 1983; Richmond & Clemens, 1985a).

By comparing the ability of mecamylamine to reduce or prevent sexual receptivity in several paradigms with the effectiveness of muscarinic antagonists in these same paradigms, it appears that there is a critical muscarinic

link in the neural circuit responsible for the induction and maintenance of sexual receptivity. In contrast, there does not appear to be a comparable nicotinic link. In fact, the lordosis-facilitating effect of systemically administered nicotine may be largely a pharmacological effect without relation to the normal neurochemical regulation of sexual receptivity.

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APPENDICES

APPENDIX 1: Individual lordosis quotients and summary statistics for estrogen-primed OVX rats before (PT) and 5 and 20 minutes after i.p. injection of nicotine (0, 25, 50, 100, or 200 µg/kg) (Experiment 1a).

#	Week	Nic Dose	Lordosis Quotient PT	5'	20'
162	1	0	0	0	0
163	2	0	0	0	0
165	1	0	0	0	0
166	1	0	0	0	0
167	2	0	0	0	0
168	1	0	0	0	0
176	2	0	0	0	0
179	2	0	0	0	0
181	1	0	0	0	0
MEAN	(n=9)	0	0	0	0
162	2	25	0	0	0
167	1	25	0	0	20
171	2	25	0	0	0
173	1	25	0	0	0
178	1	25	0	0	0
178	2	25	0	0	0
182	1	25	0	10	10
184	2	25	0	0	0
184	2	25	0	0	0
MEAN	(n=9)	25	0	1.1	3.3
161	1	50	0	0	0
161	2	50	0	0	0
165	2	50	0	20	10
168	2	50	0	100	20
170	1	50	0	0	0
174	1	50	0	0	0
176	1	50	0	20	0
179	1	50	0	0	0
181	1	50	0	90	0
182	1	50	0	80	0
MEAN	(n=10)	50	0	31	3
164	2	100	20	100	90
169	2	100	0	0	0
171	1	100	0	20	0
172	2	100	0	40	0
173	2	100	0	0	0
175	1	100	0	40	20
177	1	100	0	50	0
180	1	100	0	10	0
180	2	100	0	30	0
183	1	100	0	40	0
MEAN	(n=10)	100	2	33	11

Appendix 1, continued

#	Week	Nic Dose	Lordosis Quotient PT	5'	20'
163	1	200	0	0	0
164	1	200	0	90	50
166	2	200	0	90	10
169	1	200	0	0	0
170	2	200	0	0	0
172	1	200	0	60	0
174	2	200	0	0	0
175	2	200	0	80	20
177	2	200	0	70	20
183	2	200	0	50	0
MEAN	(n=10)	200	0	44	10

Summary statistics, Experiment 1a.

Nic Dose	n	Mean Lordosis Quotient (S.E.M.) Pretest	5-min test	20-min test
0 µg/kg	9	0	0	0
25 µg/kg	9	0	1.1 (1.1)	3.3 (2.4)
50 µg/kg	10	0	31.0 (13.2)	3.0 (2.1)
100 µg/kg	10	2 (2)	33.0 (9.3)	11.0 (9.0)
200 µg/kg	10	0	44.0 (12.6)	10.0 (5.2)

APPENDIX 2: Lordosis quotients of estrogen-primed OVX rats before (PT) and 5 minutes after nicotine injection (150 µg/kg): Effect of pretreatment with mecamylamine (MECA, 1 or 5 mg/kg), hexamethonium (HEX, 8 or 20 mg/kg), or atropine (ATR, 8 or 20 mg/kg). (Experiment 1d).

Week 4 : Mecamylamine (MECA) pretreatment prevented nicotine-induced lordosis

PreTx	PT	5'
Sal	20	30
Sal	0	80
Sal	0	80
Sal	0	90
Sal	10	100
MEAN	6	76
SEM	4	12

PreTx	PT	5'
MECA 1	0	0
MECA 1	0	0
MECA 1	0	0
MECA 1	0	0
Meca 1	0	0
	0	0
	-	-

PreTx	PT	5'
MECA 5	0	0
MECA 5	0	0
MECA 5	0	0
MECA 5	10	0
Meca 5	10	10
	4	2
	2.4	2

Week 5 : Hexamethonium (HEX) pretreatment reduced but did not prevent nicotine-induced lordosis

PreTx	PT	5'
Sal	0	40
Sal	0	70
Sal	0	70
Sal	0	80
Sal	0	100
MEAN	0	72
SEM	-	9.7

PreTx	PT	5'
HEX 8	0	0
HEX 8	0	50
HEX 8	0	60
HEX 8	0	60
HEX 8	0	60
	0	46
	-	11.7

PreTx	PT	5'
HEX 20	0	10
HEX 20	0	10
HEX 20	0	20
HEX 20	50	90
HEX 20	30	80
	16	42
	10.3	17.7

Week 6 : Atropine (Atr) pretreatment reduced but did not prevent nicotine-induced lordosis

PreTx	PT	5'
Sal	0	60
Sal	0	70
Sal	0	80
Sal	10	80
Sal	0	100
MEAN	2	78
SEM	2	6.6

PreTx	PT	5'
Atr 8	0	10
Atr 8	0	10
Atr 8	0	60
Atr 8	0	60
Atr 8	0	100
	0	48
	-	17.1

PreTx	PT	5'
Atr 20	0	10
Atr 20	0	10
Atr 20	0	40
Atr 20	0	50
Atr 20	0	60
	0	34
	-	10.3

APPENDIX 3: Lordosis quotients of estrogen-primed OVX rats before (PT) and 5 minutes after nicotine injection (150 µg/kg): Effect of pretreatment with atropine sulfate (7.5 or 30 mg/kg). (Experiment 3).

	Pretreatment (30 minutes before nicotine)					
Animal	Saline		Atropine (7.5)		Atropine (30)	
Number	PT	5'	PT	5'	PT	5'
288	0	30	0	0	0	10
289	0	30	0	50	0	0
291	0	40	0	20	0	10
292	0	100	0	50	0	30
293	0	20	0	70	0	10
294	0	50	0	0	20	10
295	0	0	0	0	0	10
297	30	80	0	70	0	0
298	0	20	0	0	0	50
299	0	90	0	0	0	10
MEAN	3	46	0	26	2	14
SEM	3	10.6	-	9.9	2	4.8