EVIDENCE FOR AN ALTERNATIVE TO THE HYPOTHESIS THAT RESERPINE FACILITATES LORDOSIS IN FEMALE RATS BY DEPLETING BRAIN SEROTONIN

by

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Chapter I

INTRODUCTION

The induction of behavioral estrus in the female of many mammalian species (e.g., rat, mouse, hamster, guinea pig, cow, ewe) is regulated by the synergistic action of the two steroid hormones: estrogen and progesterone (Young, 1961). Atypical or incomplete responses indicative of estrus can sometimes be elicited following injections of estrogen only in the spayed female. However, injection of both hormones is required to effectively induce the behavioral characteristics shown by the intact cycling animal at the time of estrus. If both hormones are required to restore the behavior following ovariectomy, it would be logical to expect that the synergistic action of both hormones operate to bring the behavior to expression in the intact female, and evidence exists to support this contention. The peak concentration of progesterone in the plasma is found to coincide with the onset of spontaneous estrus in intact rats and guinea pigs (Feder, Resko & Goy, 1968a,b), and estrus can be advanced by injection of progesterone prior to its expected onset in intact rats (Zucker, 1967), guinea pigs (Dempsey, Hertz & Young, 1936), and hamsters (Lisk, 1969).

Following the induction of heat, by either endogenous or exogenous hormones, the female rat and guinea pig will display characteristic behavior patterns which are never displayed at other times of the estrous cycle or without hormone replacement in the spayed animal.

The most characteristic estrous response is the display of lordosis (standing in place with depression of the lumbar and elevation of the sacrococcygeal region) which is displayed to either mounting attempts by the male animal or stroking of these areas by the experimenter's hand. Attempts to elicit lordosis from the anestrous female typically results in rejection behaviors such as kicking or running away. In addition to lordosis, increases in male-like mounting behavior and (for the rat) a marked increase in running activity are also characteristic of the estrous period.

Although the induction of behavioral estrus can be brought about in spayed, estrogen-primed rodents by other 21 carbon steroids such as corticosterone and desoxycorticosterone (Byrnes & Shipley, 1955) or pregnenolone and 20$_{\alpha}$-OH-pregn-4-en-3-one (Kincl, 1964; Zucker & Goy, 1967), the responses obtained are typically weaker and/or the amounts of hormone required are greater than for progesterone. Thus the induction of heat shows a relatively strong specificity for progesterone.

In a series of experiments investigating possible relationships between cerebral monoamine levels and hormone-induced estrous behavior in the spayed rat (Meyerson, 1964a,b,c), it was found that if the tranquilizer, reserpine, was injected into the estrogen-primed female, sexual receptivity (as measured by the display of lordosis) would follow (Meyerson, 1964b). Since reserpine is a non-steroidal compound, this finding appeared to challenge the well-known specific relationship between the behavior and progesterone. The hypothesis advanced to

explain this finding was that reserpine, a depletor of monoamines centrally and peripherally, was acting to remove cerebral monoamine-dependent inhibition of estrous behavior (Meyerson, 1964b). Thus, according to this hypothesis, reserpine acts directly upon the neural tissues involved in regulating the induction of sexual receptivity and that, in this sense, it substitutes for the action of progesterone upon the estrogen-primed neural substrate.

Reserpine also brings about secretion of adrenal cortical steroids via release of ACTH from the pituitary (Maickel, Westermann, & Brodie, 1961; Montanari & Stockham, 1962; Westermann, Maickel, & Brodie, 1962). However, the possibility was rejected that the behavior-inducing effect of reserpine might be due to release of some progestational steroids from the adrenal cortex because of the finding that 33% of a group of adrenalectomized-ovariectomized rats displayed lordosis following estrogen and reserpine treatment (Meyerson, 1964b).

Recent advances in techniques for the direct measurement of endogenous steroid levels have allowed for evidence to be obtained that progesterone is, under certain circumstances, secreted in measurable quantities from the adrenal cortex. Progesterone can be measured in the plasma of ovariectomized rats even 25 days after the operation. However, if female rats are simultaneously ovariectomized and adrenalectomized, progesterone concentrations fall to nondetectable levels within eight hours (Feder et al., 1968a). The concentration of progesterone in the plasma of spayed rats can be augmented by injection of ACTH but not by injection of LH (Resko, 1969). Moreover, if spayed

rats are primed with estrogen and subsequently injected with ACTH, not only are increases in concentration of progesterone in plasma found, but the display of lordosis can be measured after a predictable interval (Feder & Ruf, 1969). Thus, under conditions of ACTH stimulation the adrenal of the spayed rat can apparently secrete sufficient quantities of progesterone to act synergistically with estrogen in bringing about the induction of behavioral estrus.

The above three lines of evidence can be summarized and restated as follows: 1) reserpine stimulates release of ACTH; 2) the ACTH-stimulated adrenal secretes progesterone; and 3) lordosis can be measured following ACTH injection in the spayed, estrogen-primed rat. These known relationships suggest an alternative to Meyerson's hypothesis regarding the reserpine induction of receptivity. The alternative proposed is that injection of reserpine in the spayed, estrogen-primed rat results in elevated concentrations of progesterone in plasma of adrenal origin, and that the progesterone acts synergistically with the previously injected estrogen to induce behavioral receptivity.

Three types of experiments have been carried out to test this alternative hypothesis. In the first instance, spayed rats treated with estrogen and reserpine were tested for lordosis and bled following heat onset so that concentrations of progesterone in plasma could be measured and compared with suitable controls. These initial studies served only to demonstrate that reserpine reliably influences both the concentration of progesterone and sexual behavior.

A second series of experiments was performed in order to obtain evidence that procedures which reduce or block ACTH release and

secretion of adrenal steroids following reserpine treatment would result in significant decreases in both the concentration of progesterone in plasma and the proportion of females displaying lordosis. The direct approach of adrenalectomy was avoided in these experiments for several reasons. First, Meyerson (1964b) found that spayed-adrenalectomized females could not tolerate the dosages of reserpine which effectively induce lordosis in a majority of spayed animals. Secondly, Ring (1945) found that spayed-adrenalectomized rats showed differences from spayed females in their behavioral responsiveness to estrogen. Regenerated or accessory adrenal cortical tissue was found in most of the animals which displayed lordosis to estrogen only (Ring, 1945). Finally, Davidson, Rodgers, Smith, & Bloch (1968) presented data which suggest that the neural mechanisms for lordosis may be more sensitive to injections of estrogen in spayed-adrenalectomized rats than in rats which were only spayed.

With these problems associated with adrenalectomy in mind, alternative methods of blocking or reducing ACTH release and secretion of adrenal cortical hormones were utilized. Three different procedures were employed to achieve this end, which are believed to represent distinct methods of blocking or reducing the corticotrophin response to stress. First, animals were injected, prior to reserpine treatment, with dexamethasone, a potent corticotrophin suppressor. This compound is believed to exert its effect on the negative feedback area (median eminence) of the hypothalamus which regulates gonadotrophin secretion (Kendall, Matsuda, Duyck, & Greer, 1964). Secondly, animals were

hypophysectomized which removes the source of adrenocorticotrophic hormone. The third procedure consisted of comparing the responses, to reserpine, of female rats housed in pairs to those housed individually. Rats which are housed in small groups show an attenuated adrenocortical response to stress compared to those housed individually (Moore, 1968; Grota, Personal Communication, 1971). This phenomenon would presumably involve other brain areas which participate in regulating corticotrophin and are responsive to environmental and social stimuli. By using three distinct means of decreasing, eliminating, or attenuating adrenal stimulation, it was hoped that the generality of the results would be enhanced.

The purpose of the third experiment was to obtain evidence that corticosterone is not a significant participating steroid in the induction of lordosis under conditions of endogenous ACTH release. Metopirone is an 11β-hydroxylase inhibitor which stimulates corticotrophin release by blocking corticosterone secretion. As in the other experiments, the effects of this compound administered to estrogen-primed, spayed rats upon concentration of progesterone in plasma and upon the display of lordosis were measured.

Chapter II

METHODS

Conditions of Maintenance

The animals were housed individually (except as noted below) in 9-1/2 x 7 x 7 inch cages. An artificial light cycle (12 on - 12 off) was maintained. The light phase began at midnight. Purina rat chow and water were continuously available. The temperature was maintained at 75°F.

Subjects

Subjects were ovariectomized Sprague-Dawley rats weighing 220 - 320 grams and selected for behavioral responsiveness to estrogen and progesterone in the following way. Five to 10 days following ovariectomy, the females were subcutaneously injected with 2.5 µg estradiol benzoate at 8 to 9 PM followed 36 hr later by 0.4 mg progesterone. Five tests for lordosis were given at two-hour intervals following progesterone. Only those females which displayed lordosis within an eight-hour period following the progesterone injection were used further as subjects. This selection test provided assurance that all animals being studied would display behavioral heat following conventional replacement therapy.

Treatments and Procedural Details

Seven to 10 days after the initial selection test, all animals in the various experimental groups were injected with 2.5 µg estradiol benzoate at 8 to 9 PM. Any female that was displaying lordosis 36 hr following estradiol benzoate alone was eliminated from the study. This was done in an attempt to increase the proportion of positive cases of lordosis attributable to an interaction of estradiol with the

compound being evaluated rather than to the estradiol alone. Twenty-nine per cent displayed lordosis and were eliminated. The remainder, which had not shown lordosis to estrogen alone, were injected immediately with one of several compounds, tested hourly for the appearance of lordosis, and sacrificed for progesterone assay at a predetermined time. The specific treatment for each group of females is given at the beginning of the results section for each of the three experiments.

Methods of Testing and Measurement of Lordosis

On the test when various compounds were injected, the animals were checked hourly for the display of lordosis to manual stimulation beginning at the 36th hr following estradiol benzoate and continuing up to the time of sacrifice. Each animal was removed from its home cage at the time of each hourly test, placed in a washtub containing woodchips as bedding, and allowed to explore for 30 sec. The hair on the dorsal surface near the tail was stroked three or four times followed by brief stimulation of the anogenital region with the middle and index fingers while the thumb was held firmly across the dorsal surface. This pattern of manual stimulation was repeated five times at approximately 15-sec intervals. The lordosis response, which can be elicited by this method of manual stimulation, is characterized by assumption of a rigid posture with arching of the back and elevation of the head and tail. Three measures of behavioral response are reported in the results: 1) the percentage of animals displaying lordosis; 2) the lordosis frequency (the number of responses elicited by the five consecutive manual stimulations); and 3) latency to onset of heat.

The first two of these measures were based on the animal's performance at the last hourly check before sacrifice so that the progesterone concentrations in the plasma at the time of sacrifice could be related to the most current evaluation of the behavior.

Methods for Assay of Progesterone

The blood obtained at sacrifice was centrifuged and the plasma was removed and stored at -16°C until analyzed for progesterone content by gas-liquid chromatography. The steroid was extracted from the plasma with ether and washed with distilled water. The ether extract was dried down and concentrated to the tip of the tube under nitrogen. The residue was chromatographed on thin layer in benzene-ethylacetate (2:1). Areas corresponding to authentic progesterone were scraped off and extracted with 95% methanol. The extract was subjected to enzymatic conversion to 20β-OH-pregn-4-en-3-one followed by chloracetylation. The chloracetate derivative of 20β-OH-pregn-4-en-3-one was isolated by thin layer chromatography, extracted with benzene, and measured by a Barber Colman gas chromatograph. The per cent recovery of progesterone from the extraction procedure was calculated by adding approximately 5,000 cpm of tritiated progesterone (specific activity = 45.8 millicuries/millimole) to the plasmas prior to extraction. One-tenth aliquot of the benzene extract was removed for determination of recovery of tritiated progesterone by a Packard liquid scintillation spectrometer. A standard of testosterone monochloracetate was added to the benzene extract to calculate per cent recovery on the chromatograph column. The benzene extract was dried down and concentrated

under nitrogen. The residue was injected into the column in 15 µl toluene. The areas of chromatographic peaks for 20β-OH-pregn-4-en-3-one chloracetate were measured and quantitated by a standard curve derived from chromatographic peak areas corresponding to known amounts of 20β-OH-pregn-4-en-3-one chloracetate injected into the gas chromatograph.

Chapter III

RESULTS

Experiment 1. Demonstration that reserpine increases the concentration of progesterone in plasma and facilitates lordosis.

Treatment. Estrogen-primed females were injected at Hr 36 with either 0.5 mg reserpine (Serpasil, Ciba, diluted with distilled water to 1 mg/ml) or 0.5 cc distilled water (controls). The animals were randomly selected for sacrifice following the 5th and 6th hourly check for lordosis. At these times they were injected with nembutal* (35 mg/kg), and blood was collected by cardiac exsanguination.

Results. Lordosis was displayed by 100% of the estrogen-primed females following an injection of 0.5 mg reserpine (Table 1). The mean latency to the first lordosis response was 3.5 hr (SD = 0.94). The median frequency of lordosis responses elicited from these animals was 3; six animals had scores of 3 or less, while the other five females displayed lordosis 4 or more times on the last hourly check. In contrast to reserpine-treated animals, only 30% of control animals displayed lordosis. These proportions were significantly different by a Fisher's exact test ($p = .0010$).

The concentration of progesterone in plasma was significantly higher following reserpine injection than following a control injection of distilled water ($U = 1$, $p < .001$). The median concentration of progesterone recovered from the systemic plasma of reserpine-treated rats was 4.6 ng/ml; for controls the median value was 1.4 ng/ml (Table 1).

*Pentobarbital sodium

Table 1

The Display of Lordosis and Concentrations of Progesterone in the Systemic Plasma of Spayed, Estrogen-Primed Rats Following Treatment with Reserpine

Treatment	No. of Rats	% Animals Showing Lordosis		Lordosis Frequency (Median & Range)	Concentrations of Progesterone (ng/ml) in Plasma (Median & Range)	
0.5 mg Reserpine	11	100	p=.0010	3 (2-5)	4.6 (2.9-8.6)	p<.001
0.5 cc H_2O (Control)	10	30		0 (0-5)	1.4 (ND*-3.1)	

*Concentration too low for detection (nondetectable).

Experiment 2. Effect of procedures which prevent or reduce adrenal cortical secretions following reserpine treatment.

Treatments. Six groups of estrogen-primed females were used for this experiment. The first two groups were injected with 0.75 mg reserpine at Hr 36 following estrogen. The second group received, in addition, an injection of 500 μg/kg body wt dexamethasone (9α-fluoro-11β, 17α, 21-trihydroxy-16α-methyl-1,4-pregnadiene-3, 20-dione) at Hr 35, i.e. 1 hr prior to reserpine treatment. The third group of animals also received an injection of dexamethasone (100 or 500 μg/kg) at Hr 35 but was injected with 0.4 mg progesterone at Hr 36. For the fourth group, animals hypophysectomized two weeks previously were injected at Hr 36 with 0.75 mg reserpine. The fifth and sixth groups were injected with 0.5 mg reserpine at Hr 36. For one of these groups, females were housed two to a cage, while for the other group females were housed individually. Sacrifices and blood collections were as in experiment 1.

Results. The proportion of females displaying lordosis and the concentration of progesterone in plasma were lower in females treated with dexamethasone 1 hr before reserpine than in females given reserpine alone (Table 2). When animals were injected with 0.75 mg reserpine, lordosis was subsequently displayed by 83% of the animals with a mean latency of 2.5 hr (SD = 1.08) and a median lordosis frequency of 4. When the same amount of reserpine was given to animals which received an injection of 500 μg/kg body wt dexamethasone 1 hr previously, only 22% displayed lordosis. The difference in proportion of animals showing lordosis was significant ($p = .0084$). The median concentration

Table 2

Effect of Dexamethasone on Concentration of Progesterone in Systemic Plasma and the Display of Lordosis Following Treatment with Reserpine

Treatment	No. of Rats	% Animals Showing Lordosis		Lordosis Frequency (Median & Range)	Concentration of Progesterone (ng/ml) in Plasma (Median & Range)	
0.75 mg Reserpine	12	83	p=.0084	4 (0-5)	5.5 (Tr*-9.2)	p<.001
500 µg/kg Dexamethasone + 0.75 mg Reserpine	9	22		0 (0-4)	0.8 (ND**-1.6)	
100 or 500 µg/kg Dexamethasone + 0.4 mg Progesterone	9	100		5 (5-5)	--	

* Concentration too low for quantification (trace).

** Concentration too low for detection (nondetectable).

of progesterone in the plasmas of animals injected with 0.75 mg reserpine was 5.5 ng/ml, while for animals injected with dexamethasone prior to reserpine the median concentration was only 0.8 ng/ml (Table 2). The difference in plasma progesterone concentration between the two groups was also significant ($U = 7$, $p < .001$). Thus, in spite of injection with a relatively high dose of reserpine, lordosis was not displayed by a high proportion of females in the absence of progesterone in the plasma.

Dexamethasone did not interfere with the display of lordosis induced by estrogen and progesterone. When nine spayed rats were injected with 100 or 500 μg/kg body wt dexamethasone 1 hr prior to progesterone at Hr 36, all females came into behavioral heat and the median lordosis frequency was 5. Neither the percentage of animals responding nor the median lordosis frequency was different under these conditions of treatment than from the corresponding values obtained from the same females previously tested with estradiol benzoate and progesterone only, i.e. on their initial selection test. Thus, although dexamethasone did not interfere with the responsiveness of the lordosis mechanism to estrogen followed by progesterone, the same compound prevented the display of lordosis in females treated with estradiol benzoate followed by reserpine.

Further evidence in addition to that obtained with dexamethasone indicated that the ability of reserpine to elevate plasma levels of progesterone depended upon the endogenous release of ACTH. Four hypophysectomized, spayed female rats were treated with 2.5 μg

estradiol benzoate followed by 0.75 mg reserpine 36 hr later. The animals were killed 5.0 hr following the injection of reserpine, and in every case the concentration of progesterone in the plasma was lower than that detectable by our methods. Unlike the animals pretreated with dexamethasone, these animals could not be used to show that lordosis failed to occur in the absence of measurable quantities of progesterone in the plasma following reserpine treatment. The animals were unsuitable for this purpose inasmuch as all four of the subjects were displaying lordosis immediately prior to the time reserpine was injected. For reasons not now clear, these hypophysectomized females displayed an unexpectedly high behavioral sensitivity to estrogen treatment alone.

A third study in this experiment provided additional evidence indicating that reduced concentrations of progesterone in plasma following reserpine treatment are associated with a reduction in the display of lordosis. When estrogen-primed females which were housed two to a cage were injected with 0.5 mg reserpine, significantly fewer animals displayed lordosis than did females receiving the same treatment but housed individually ($p = .0350$)(Table 3). Although the median concentration of progesterone recovered from the plasmas of these animals was 4.1 ng/ml and Tr for females housed individually or in pairs respectively, a Mann-Whitney U test failed to show a significant difference between the groups ($U = 13$, $p = 0.08$). However, if progesterone determinations of nondetectable and trace are assigned values of 0 and 0.1 ng/ml respectively, the means are 4.4 ng/ml for

Table 3

Effects of Housing Conditions on the Display of Lordosis and the Concentration of Progesterone in Systemic Plasma of Estrogen-Primed Spayed Rats Following Treatment with Reserpine

Treatment	No. of Rats	% Animals Showing Lordosis		Lordosis Frequency (Median & Range)	Concentration of Progesterone (ng/ml) in Plasma (Mean & Standard Deviation)	
0.5 mg Reserpine, individually housed	7	100	p=.0350	4 (2-5)	4.4 (± 1.99)	p<.01
0.5 mg Reserpine, doubly housed	7	43		0 (0-5)	1.6 (± 1.24)	

individually housed animals and 1.6 ng/ml for those housed in pairs and a t-test reveals a significant difference ($t = 3.9$, $df = 12$, $p < .01$)(Table 3).

Experiment 3. Effect of blocking corticosterone synthesis with Metopirone on the display of lordosis and concentrations of progesterone in plasma.

Treatment. Two groups of estrogen-primed animals were injected with 100 mg/kg body wt Metopirone (Ciba, 2-methyl-1,2-di-3-pyridyl-1-propanone). One of the groups received dexamethasone (500 µg/kg) 1 hr prior to the injection of Metopirone. The animals were sacrificed as above, except that the time of sacrifice (following the 6th and 7th hourly checks for lordosis) was different. The change in the time of sacrifice was introduced for this experiment because preliminary data indicated that the latent period to the onset of heat was longer following Metopirone than following reserpine. Thus, in order to collect blood from animals in a comparable behavioral state, the time of sacrifice had to be altered.

Results. When levels of corticosterone were reduced by injection of Metopirone* into spayed, estrogen-primed rats, 78% of the animals displayed lordosis with a mean latency of 5.3 hr (SD = 2.36) and a

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* Corticosterone was measured in 4 of the Metopirone-treated animals and in 5 controls injected with distilled water. The median values of corticosterone recovered from the plasmas of these animals were 25 and 54 µl/100 ml, respectively. The Metopirone-treated animals showed significantly lower levels of corticosterone ($U = 0.5$, $p < .01$). The corticosterone was measured fluorometrically by Mr. Gus Abro in Dr. F. Robert Brush's laboratory at The University of Oregon Medical School.

median lordosis frequency of 4 (Table 4). The median concentration of progesterone recovered from the plasmas of these animals was 4.2 ng/ml (Table 4). Neither the proportion of animals showing lordosis nor the concentrations of progesterone in plasma were different following Metopirone treatment than the corresponding values obtained from animals treated with reserpine (0.5 and 0.75 mg) in experiments 1 and 2 ($X^2 = 1.08$, $p > 0.1$, $df = 1$; $U = 96$, $Z = 0.33$, $p > 0.1$, respectively). When dexamethasone was injected 1 hr prior to Metopirone only 20% of the animals displayed lordosis and the concentration of progesterone in the plasma was nondetectable for all animals except one which showed a trace amount (Table 4). The differences in proportion of animals responding and in plasma concentrations of progesterone between the two groups were significant ($p = .0185$, $U = 0.5$, $p < .001$, respectively). These results parallel those of experiment 2 in the sense that when the expected increase in the concentration of progesterone in plasma was prevented by pretreatment with dexamethasone, lordosis was not displayed by the majority of animals.

Table 4

Induction of Lordosis and Concentration of Progesterone in Systemic Plasma Following Treatment with Metopirone or Dexamethasone plus Metopirone in the Spayed, Estrogen-Primed Rat

Treatment	No. of Rats	% Animals Showing Lordosis		Lordosis Frequency (Median & Range)	Concentration of Progesterone (ng/ml) in Plasma (Median & Range)	
100 mg/kg Metopirone	9	78	p=.0185	4 (0-5)	4.2 (Tr*-8.7)	p<.001
500 µg/kg Dexamethasone + 100 mg/kg Metopirone	10	20		0 (0-3)	ND** (ND-Tr)	

* Concentration too low for quantification (trace).

** Concentration too low for detection (nondetectable).

Chapter IV

DISCUSSION

In the present experiments a strong positive relationship was found between the display of the lordosis response and the endogenous levels of progesterone recovered from the plasma. When reserpine brought about elevated concentrations of progesterone in plasma, a high proportion of females displayed lordosis. In contrast, the levels of progesterone recovered from the plasmas of control animals injected with distilled water were significantly lower, and the proportion of control females showing lordosis was significantly smaller. When the increase in progesterone concentration in plasma following reserpine treatment was reduced to below control levels by pretreatment with dexamethasone, the proportion of animals displaying lordosis was correspondingly reduced to below control levels. This latter finding of decreased levels of progesterone when dexamethasone was injected prior to reserpine as well as the inability to recover detectable quantities of progesterone from the plasma of hypophysectomized females following reserpine treatment provides evidence that the source of the progesterone is the adrenal cortex. Finally, when animals were housed in pairs so that resistance to the stressor actions of injected reserpine was increased, both the concentration of progesterone in plasma and the proportion of animals displaying lordosis were low compared with animals housed individually. These results are consistent with, and lend support to, the hypothesis that reserpine brings about the display of lordosis by its effects on ACTH release and the secretion of progesterone from the adrenal.

Corticosterone is probably not a significant participating steroid in the induction of heat under conditions of endogenous ACTH release. When the normally high levels of corticosterone associated with adrenal stimulation were prevented by Metopirone treatment (resulting in compensatory ACTH release), the concentrations of progesterone in plasma and the percentage of females showing lordosis were no different than following treatment with reserpine. In addition, unpublished experiments by the author revealed that a small percentage of animals (20%) displayed lordosis following an injection of corticosterone which was 50 times an effective dose of progesterone. Desoxycorticosterone could possibly have a summating effect with progesterone in bringing about the behavior; however, this compound was also much less effective than progesterone when tested in spayed, estrogen-primed females.

Since a variety of experimental procedures have been shown to produce a stress response, i.e. adrenocortical activation (Barrett & Stockham, 1963; Moore, 1968; Grota, Personal Communication, 1971), it may be wondered why the stress of handling the animals is not sufficient to induce lordosis. The experimental manipulations (injection at Hr 36 and removal from home cage for hourly tests) were, in fact, followed by lordosis in a small proportion of control animals. The magnitude of the adrenocortical response, as measured by progesterone, was, however, much less following control injection than following reserpine even though animals were treated identically in all other respects. Thus, it may be that a certain threshold amount of progesterone is required to effectively induce lordosis in most animals. In addition, differences in duration of the adrenocortical responses may exist (Ader, 1970; Grota,

Personal Communication, 1971). The neurochemical changes, initiated by progesterone, which bring about the expression of the behavior may require the continuous presence of the hormone for a period of several hours. Reserpine has been shown to cause prolonged hypersecretion of ACTH (up to 8 hr) similar to that produced by continuous exposure to cold stress (Maickel et al., 1961). The animals' adrenocortical response to the stress of handling, however, might be of short duration. These two factors, magnitude and duration of the adrenocortical response, may account for the absence of lordosis in the majority of control females.

Attempts to correlate changes in brain levels of the biogenic amines with sexual behavior and gonadotrophin regulation have yielded inconsistent and sometimes contradictory results. Even though Meyerson (1964c) claimed that serotonin depletion was important for lordosis, he was unable to measure depletion of serotonin following estrogen and progesterone treatment, i.e. the two hormones which operate synergistically to bring the behavior to expression. Attempts to correlate selective depletion of brain serotonin induced by para-chlorophenylalanine with sex behavior has resulted in some investigators claiming a facilitation of sexual responses (Tagliamonte, Tagliamonte, Gessa, & Brodie, 1969; Sheard, 1969; Shillito, 1970; Ferguson, Henriksen, Cohen, Mitchell, Barchas, & Dement, 1970; Meyerson & Lewander, 1970; Sjoerdsma, Lovenberg, Engelman, Carpenter, Wyatt, & Gessa, 1970) while others report no change or a slight inhibitory effect (Whalen & Luttge, 1970; Segal & Whalen, 1970; Zitrin, Beach, Barchas, & Dement, 1970). Changes in other brain mono-

amines (norepinephrine and dopamine) have also been measured in various reproductive states and following hormone treatment. Stefano and Donoso (1967) report changes in hypothalamic concentration of norepinephrine throughout the vaginal estrous cycle of rats, whereas Sandler (1968) was unable to detect any such changes. In addition, Donoso and Stefano (1967) found that injections of estrogen and progesterone in amounts that were sufficient to inhibit gonadotrophin release in castrated rats did not have a significant effect on the hypothalamic concentration of norepinephrine. Clearly, changes in brain levels of monoamines have not been associated unambiguously with either the regulation of sexual behavior or gonadotrophin secretion. The present results indicate that the effects of reserpine on female sex behavior can be adequately explained by the secretion of progesterone from the adrenal cortex and a hypothesis of monoamine depletion need not be involved.

A final argument that the effects of reserpine on lordosis (and probably on other reproductive processes) are mediated by the secretion of progesterone from the adrenal can be derived by a survey of the literature which shows that the majority of effects with reserpine (in the rat) can be duplicated with progesterone. Exogenous progesterone (Everett, 1948) as well as reserpine (Barraclough & Sawyer, 1957) will inhibit ovulation in the rat if given at appropriate times. A single injection of progesterone (Alloiteau & Vignal, 1958; Everett, 1963; Rothchild & Schubert, 1963) or reserpine (Barraclough & Sawyer, 1959) during estrus will induce pseudopregnancy; and both reserpine and

progesterone (Meites, Nicoll, & Talwalker, 1963; Cowie & Folley, 1961) will influence mammary gland function and presumably pituitary prolactin production in the rat. Both compounds will maintain pregnancy in rats receiving a protein-free diet (Nelson & Evans, 1955; Kalivas & Nelson, 1966) as well as offer protection against the impairment of ovarian function induced by androgen in the neonatal rat (Kikuyama, 1961; Cagnoni, Fantani, Morace, & Ghetti, 1965; Kincl & Maqueo, 1965; Arai & Gorski, 1968). Finally, both reserpine and progesterone induce sexual receptivity in the spayed, estrogen-primed rat (Meyerson, 1964a). The explanation of these parallels does not require a hypothesis of similar mechanisms of action for reserpine and progesterone on brain levels of monoamines. On the contrary, the present results suggest that some effects of reserpine can be explained by its ability to trigger the release of ACTH which stimulates the adrenal gland to secrete progesterone and possibly other steroids with physiological actions which may potentiate those of progesterone.

The research reported in this thesis and most of the literature cited has been limited to data on the rat. Reserpine may have quite different effects in other species which may or may not depend on activation of ACTH release and secretion of adrenal steroids. In a recent report (Uphouse, Wilson, & Schlesinger, 1970), reserpine was found to stimulate lordosis in the spayed mouse but was not effective in spayed-adrenalectomized mice. These authors reached a similar conclusion to that presented in this thesis, i.e. that lordosis is stimulated by secretion of adrenal hormones following reserpine

treatment. Quite different results have been obtained, however, with female guinea pigs and reserpine (Paris, unpublished results). The display of lordosis was found not to be facilitated and progesterone was not detectable in plasma following reserpine treatment in spayed, estrogen-primed guinea pigs. On the contrary, the display of lordosis was inhibited by reserpine in this species even when adequate amounts of progesterone were injected into estrogen-primed animals. In the rhesus monkey, reserpine has been found to lengthen the menstrual cycle and cause uterine bleeding following withdrawal of the drug when injected during the follicular phase of the cycle (Erikson, 1969). More studies are needed to determine not only if the effects of reserpine in other species are mediated by ACTH release and adrenal cortical hormone secretion, but also to attempt to identify the types of adrenal hormones predominantly involved. Such studies, if effectively carried out, might be expected to lead to new hypotheses about the ways in which stress influences the reproductive cycles of higher primates.

Chapter V

SUMMARY

Three experiments were carried out to test the hypothesis that reserpine facilitates lordosis in spayed, estrogen-primed rats by releasing ACTH which stimulates the secretion of progesterone from the adrenal cortex. The progesterone then is believed to act synergistically with estrogen in inducing lordosis. In the first experiment, spayed, estrogen-primed rats were injected with reserpine or distilled water (controls), tested hourly for lordosis, and killed following heat onset so that concentration of progesterone in plasma could be measured by gas-liquid chromatography. The proportion of females displaying lordosis and the concentration of progesterone in plasma were significantly greater following reserpine injection than following control injection.

The second experiment studied the effects of three different procedures aimed at preventing or reducing the release of ACTH and secretion of adrenal steroids following reserpine treatment. Injection of dexamethasone prior to reserpine greatly reduced both the concentration of progesterone in plasma and the proportion of females displaying lordosis. Animals housed in pairs and treated with reserpine also showed reductions in both the concentration of progesterone in plasma and the proportion of animals displaying lordosis compared to those housed individually. Progesterone was not detectable in plasma of hypophysectomized females following treatment with reserpine.

The third experiment attempted to demonstrate that corticosterone is not a significant participating steroid in the induction of heat

under conditions of endogenous ACTH release. Females injected with Metopirone, which blocks synthesis of corticosterone, had progesterone levels comparable to and displayed lordosis in proportion similar to females treated with reserpine. Dexamethasone given prior to Metopirone prevented the expected elevation of progesterone in plasma and reduced the number of females displaying lordosis.

It is concluded that reserpine facilitates lordosis in the spayed, estrogen-primed rat, not by altering the concentration of monoamines in neural tissues involved in regulating the behavior, but indirectly by its effects on ACTH release and secretion of progesterone from the adrenal. Accordingly, the view that reserpine induces lordosis is held to be incorrect. The evidence indicates that the lordosis observed in estrogen-primed rats following reserpine is brought to expression by the usual estrogen-progesterone synergy and no less parsimonious explanation need be sought.

REFERENCES

Ader, R. The effects of early experience on the adrenocortical response to different magnitudes of stimulation. Physiol. Behav., 1970. 5, 837-839.

Alloiteau, J.J., & Vignal, A. Pseudogestation après injection de progestérone chez la ratte. Compt. Rend. Acad. Sci., 1958. 246, 2804-2807.

Arai, Y., & Gorski, R.A. Protection against the neural organizing effect of exogenous androgen in the neonatal female rat. Endocrinology, 1968. 82, 1005-1009.

Barraclough, C.A., & Sawyer, C.H. Blockade of the release of pituitary ovulating hormone in the rat by chlorpromazine and reserpine: possible mechanisms of action. Endocrinology, 1957. 61, 341-351.

Barraclough, C.A., & Sawyer, C.H. Induction of pseudopregnancy in the rat by reserpine and chlorpromazine. Endocrinology, 1959. 65, 563-571.

Barrett, A.M., & Stockham, M.A. The effect of housing conditions and simple experimental procedures upon the corticosterone level in the plasma of rats. J. Endocrinol., 1963. 26, 97-105.

Byrnes, W.W., & Shipley, E.G. Guinea pig copulatory reflex in response to adrenal steroids and similar compounds. Endocrinology, 1955. 57, 5-9.

Cagnoni, M., Fantini, F., Morace, G., & Ghetti, A. Failure of testosterone propionate to induce the 'early-androgen' syndrome in rats previously injected with progesterone. J. Endocrinol., 1965. 33, 527-528.

Cowie, A.T., & Folley, S.J. The mammary gland and lactation. In W.C. Young (Ed.) Sex and internal secretions, Vol. 1. Baltimore: Williams and Wilkins, 1961. pp. 590-642.

Davidson, J.M., Rodgers, C.H., Smith, E.R., & Bloch, G.H. Stimulation of female sex behavior in adrenalectomized rats with estrogen alone. Endocrinology, 1968. 82, 193-195.

Dempsey, E.W., Hertz, R., & Young, W.C. The experimental induction of oestrus (sexual receptivity) in the normal and ovariectomized guinea pig. Am. J. Physiol., 1936. 116, 201-209.

Donoso, A.O., & Stefano, F.J.E. Sex hormones and concentration of noradrenalin and dopamine in the anterior hypothalamus of castrated rats. Experientia, 1967. 23, 665-666.

Erikson, L.B. The comparative effect of reserpine and carbethoxy-syringoyl methylreserpate (Su 3118) upon the menstrual cycle of the rhesus monkey. Canad. J. Physiol. Pharmacol., 1969. 47, 99-104.

Everett, J.W. Progesterone and estrogen in the experimental control of ovulation time and other features of the estrous cycle in the rat. Endocrinology, 1948. 43, 389-405.

Everett, J.W. Pseudopregnancy in the rat from brief treatment with progesterone: effect of isolation. Nature, 1963. 198, 695-696.

Feder, H.H., Resko, J.A., & Goy, R.W. Progesterone levels in the arterial plasma of pre-ovulatory and ovariectomized rats. J. Endocrinol., 1968a. 41, 563-569.

Feder, H.H., Resko, J.A., & Goy, R.W. Progesterone concentrations in the arterial plasma of guinea-pigs during the oestrous cycle. J. Endocrinol., 1968b. 40, 505-513.

Feder, H.H., & Ruf, K.B. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. Endocrinology, 1969. 84, 171-174.

Ferguson, J., Henriksen, S., Cohen, H., Mitchell, G., Barchas, J., & Dement, W. "Hypersexuality" and behavioral changes in cats caused by administration of p-chlorophenylalanine. Science, 1970. 168, 499-501.

Grota, L.J. Personal Communication. 1971.

Kalivas, D.T., & Nelson, M.M. Maintenance of pregnancy by reserpine in the absence of dietary protein. Endocrinology, 1966. 79, 460-462.

Kendall, J.W., Matsuda, K., Duyck, C., & Greer, M.A. Studies of the location of the receptor site for negative feedback control of ACTH release. Endocrinology, 1964. 74, 279-283.

Kikuyama, S. Inhibitory effect of reserpine on the induction of persistent estrus by sex steroids in the rat. Annot. Zool. Japon., 1961. 34, 111-116.

Kincl, F.A. Copulatory reflex response to steroids. In R.I. Dorfman (Ed.) Methods in hormone research, Vol. 3. New York: Academic Press, 1964. pp. 477-484.

Kincl, F.A., & Maqueo, M. Prevention by progesterone of steroid-induced sterility in neonatal male and female rats. Endocrinology, 1965. 77, 859-862.

Lisk, R.D. Progesterone: role in limitation of ovulation and sex behavior in mammals. Trans. N. Y. Acad. Sci., 1969. 31, 593-601.

Maickel, R.P., Westermann, E.O., & Brodie, B.B. Effects of reserpine and cold-exposure on pituitary adrenocortical function in rats. J. Pharmacol. Exp. Ther., 1961. 134, 167-175.

Meites, J., Nicoll, C.S., & Talwalker, P.K. The central nervous system and the secretion and release of prolactin. In A.V. Nalbandov (Ed.) Advances in neuroendocrinology. Urbana: University of Illinois Press, 1963. pp. 238-277.

Meyerson, B.J. The effect of neuropharmacological agents on hormone-activated estrus behaviour in ovariectomised rats. Arch. Inter. Pharmacodyn. Ther., 1964a. 150, 3-34.

Meyerson, B.J. Estrus behaviour in spayed rats after estrogen or progesterone treatment in combination with reserpine or tetrabenazine. Psychopharmacologia (Berl.), 1964b. c.6, 210-218.

Meyerson, B.J. Central nervous monoamines and hormone induced estrus behaviour in the spayed rat. Acta Physiol. Scand., 1964c. 63, Suppl. I, 241-273.

Meyerson, B.J., & Lewander, T. Serotonin synthesis inhibition and estrous behaviour in female rats. Life Sci., 1970. 9, Part I, 661-671.

Montanari, R., & Stockham, M.A. Effects of single and repeated doses of reserpine on the secretion of adrenocorticotrophic hormone. Brit. J. Pharmacol., 1962. 18, 337-345.

Moore, K.E. Studies with chronically isolated rats: tissue levels and urinary excretion of catecholamines and plasma levels of corticosterone. Canad. J. Physiol. Pharmacol., 1968. 46, 553-558.

Nelson, M.M., & Evans, H.M. Maintenance of pregnancy in absence of dietary protein with progesterone. Proc. Soc. Exp. Biol. Med., 1955. 88, 444-446.

Resko, J.A. Endocrine control of adrenal progesterone secretion in the ovariectomized rat. Science, 1969. 164, 70-71.

Ring, J.R. The hormonal induction of mating responses in the spayed-adrenalectomized female rat. Endocrinology, 1945. 37, 237-244.

Rothchild, I., & Schubert, R. Corpus luteum-pituitary relationship: induction of pseudopregnancy in the rat by progesterone. Endocrinology, 1963. 72, 969-972.

Sandler, R. Concentration of norepinephrine in the hypothalamus of the rat in relation to the estrous cycle. Endocrinology, 1968. 83, 1383-1386.

Segal, D.S., & Whalen, R.E. Effect of chronic administration of p-chlorophenylalanine on sexual receptivity of the female rat. Psychopharmacologia, 1970. 16, 434-438.

Sheard, M.H. The effect of p-chlorophenylalanine on behavior in rats: relation to brain serotonin and 5-hydroxy indole acetic acid. Brain Res., 1969. 15, 524-528.

Shillito, E.E. The effect of para-chlorophenylalanine on social interaction of male rats. Brit. J. Pharmacol. Chemother., 1970. 38, 305-315.

Sjoerdsma, A., Lovenberg, W., Engelman, K., Carpenter, W.T., Wyatt, R.J., & Gessa, G.L. Serotonin now: clinical implications of inhibiting its synthesis with para-chlorophenylalanine. Ann. Intern. Med., 1970. 73, 607-629.

Stefano, F.J.E., & Donoso, A.O. Norepinephrine levels in rat hypothalamus during the estrous cycle. Endocrinology, 1967. 81, 1405-1406.

Tagliamonte, A., Tagliamonte, P., Gessa, G.L., & Brodie, B.B. Compulsive sexual activity induced by p-chlorophenylalanine in normal and pinealectomized male rats. Science, 1969. 166, 1433-1435.

Uphouse, L.L., Wilson, J.R., & Schlesinger, K. Induction of estrus in mice: the possible role of adrenal progesterone. Hormones and Behavior, 1970. 1, 255-264.

Westermann, E.O., Maickel, R.P., & Brodie, B.B. On the mechanism of pituitary-adrenal stimulation by reserpine. J. Pharmacol. Exp. Ther., 1962. 138, 208-217.

Whalen, R.E., & Luttge, W.G. P-chlorophenylalanine methyl ester: an aphrodisiac? Science, 1970. 169, 1000-1001.

Young, W.C. The hormones and mating behavior. In W.C. Young (Ed.) Sex and internal secretions, Vol. 2. Baltimore: Williams and Wilkins, 1961. pp. 1173-1239.

Zitrin, A., Beach, F.A., Barchas, J.D., & Dement, W.C. Sexual behavior of male cats after administration of parachlorophenylalanine. Science, 1970. 170, 868-870.

Zucker, I. Progesterone in the experimental control of the behavioural sex cycle in the female rat. J. Endocrinol., 1967. 38, 269-277.

Zucker, I., & Goy, R.W. Sexual receptivity in the guinea pig: inhibitory and facilitatory actions of progesterone and related compounds. J. Comp. Physiol. Psychol., 1967. 64, 378-383.