

1987

Maintenance of progesterone-facilitated sexual behavior in female rats requires continued hypothalamic protein synthesis and nuclear progestin receptor occupation.

Michael J. Moore
University of Massachusetts Amherst

Follow this and additional works at: <https://scholarworks.umass.edu/theses>

Moore, Michael J., "Maintenance of progesterone-facilitated sexual behavior in female rats requires continued hypothalamic protein synthesis and nuclear progestin receptor occupation." (1987). *Masters Theses 1911 - February 2014*. 2115.
<https://doi.org/10.7275/7675777>

This thesis is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Masters Theses 1911 - February 2014 by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

312066014818062

MAINTENANCE OF PROGESTERONE-FACILITATED SEXUAL BEHAVIOR
IN FEMALE RATS REQUIRES CONTINUED HYPOTHALAMIC PROTEIN
SYNTHESIS AND NUCLEAR PROGESTIN RECEPTOR OCCUPATION

A Thesis Presented

By

Michael J. Moore, Jr.

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May 1987

Department of Psychology

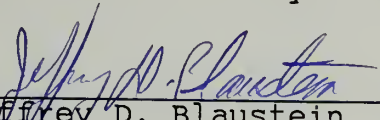
MAINTENANCE OF PROGESTERONE-FACILITATED SEXUAL BEHAVIOR
IN FEMALE RATS REQUIRES CONTINUED HYPOTHALAMIC PROTEIN
SYNTHESIS AND NUCLEAR PROGESTIN RECEPTOR OCCUPATION

A Thesis Presented

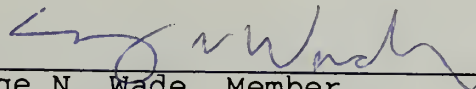
By

Michael J. Moore, Jr.

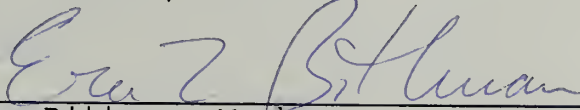
Approved as to style and content by:



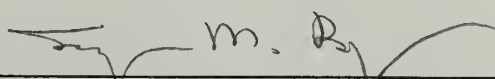
Jeffrey D. Blaustein, Chairperson of Committee



George N. Wade, Member



Eric L. Bittman, Member



Seymour M. Berger, Department Head
Department of Psychology

ACKNOWLEDGEMENTS

I wish to thank Jeff Blaustein for taking me into his lab and giving me a taste of graduate research. I also wish to thank the two other committee members, George Wade and Eric Bittman, for their helpful comments on this thesis.

I am fully indebted to both Ted Brown and Libby Swearengen. Their friendship kept me going during the first year of graduate school.

Many people at the University of Massachusetts helped to define my present feelings towards academia. Special thanks are due to: Diane Blazis, Yvon Deville, Julie Foulsham, Joan Hamilton, Joliene Hart, Ben Letcher, Deb Olster, Wendy Sue Rand, Jill Schneider, and Rachel Yehuda. Extra special thanks are due to Debbie Merritt, who was there whenever I needed her.

ABSTRACT

MAINTENANCE OF PROGESTERONE-FACILITATED SEXUAL BEHAVIOR
IN FEMALE RATS REQUIRES CONTINUED HYPOTHALAMIC PROTEIN
SYNTHESIS AND NUCLEAR PROGESTIN RECEPTOR OCCUPATION

MAY 1987

MICHAEL J. MOORE, B.S., SUFFOLK UNIVERSITY
M.S., UNIVERSITY OF MASSACHUSETTS

Directed by: Professor Jeffrey D. Blaustein

When ovariectomized rats receive sequential injections of estradiol and progesterone, the animals exhibit a period of sexual behavior which ends approximately 18 h after progesterone treatment. However, the concentration of hypothalamic nuclear progestin receptors in similarly-treated animals returns to baseline levels 8-10 h after progesterone treatment. This pattern differs from guinea pigs in which hypothalamic nuclear progestin receptor levels remain elevated throughout the period of sexual receptivity. A series of experiments examined the role of ongoing hypothalamic protein synthesis and elevated levels of nuclear-bound hypothalamic progestin receptors in maintaining the period of sexual behavior in ovariectomized rats treated with 500 μ g of progesterone 44 h after receiving 2 μ g of estradiol benzoate.

When the protein synthesis inhibitor, anisomycin (100 mg/kg body weight), was injected at 2 or 10 h after progesterone treatment, sexual behavior was blocked within

2 h. This dose of anisomycin inhibited protein synthesis in the mediobasal hypothalamus by 70% within 2 h of injection. When the progesterone antagonist, RU 486 (5 mg), was injected at 2, 6, or 10 h after progesterone treatment, sexual behavior was inhibited within 4-8 h. Using a modified receptor assay, elevated concentrations of hypothalamic nuclear progestin receptors were measured at 10 and 14 h, but not at 18 h, after progesterone injection.

These results suggest that throughout the period of female sexual behavior in rats, as was reported in guinea pigs, progesterone-facilitated sexual receptivity is maintained by an elevated level of progesterone-occupied nuclear progestin receptors in the mediobasal hypothalamus. Furthermore, these results also suggest that progesterone activates and maintains sexual behavior by inducing hypothalamic proteins that last for less than 2 h.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
LIST OF ILLUSTRATIONS	viii
Chapter	
I. INTRODUCTION	1
II. METHODS	17
Subjects	17
Tests for sexual receptivity	18
Brain dissection	19
Protein synthesis assay	20
Nuclear progesterin receptor assay	21
Statistical analysis	23
III. EXPERIMENT 1: INHIBITING PROGESTERONE- FACILITATED SEXUAL BEHAVIOR WITH ANISOMYCIN	24
Rationale	24
Procedure	25
Results	27
Protein synthesis	27
Sexual behavior - anisomycin injected 2 h after progesterone	27
Sexual behavior - anisomycin injected 10 h after progesterone	27
IV. EXPERIMENT 2: INHIBITING PROGESTERONE- FACILITATED SEXUAL BEHAVIOR WITH RU 486	32
Rationale	32
Procedure	33
Results	34
RU 486 injected 2 h after progesterone	34
RU 486 injected 6 h after progesterone	35
RU 486 injected 10 h after progesterone	35

V.	EXPERIMENT 3: DECREASES IN THE CONCENTRATION OF NUCLEAR PROGESTIN RECEPTORS OVER TIME	43
	Rationale	43
	Procedure	44
	Results	45
	Standard vs. modified cell nuclei isolation	45
	Mediobasal hypothalamus and preoptic area	45
	Anterior pituitary	45
VI.	EXPERIMENT 4: RU 486 BINDING TO NUCLEAR PROGESTIN RECEPTORS	48
	Rationale	48
	Procedure	49
	Results	49
VII.	EXPERIMENT 5: RU 486 EXCHANGE WITH R 5020 .	52
	Rationale	52
	Procedure	52
	Results	53
VIII.	DISCUSSION	54
	
	BIBLIOGRAPHY	64

LIST OF ILLUSTRATIONS

1. Effect of anisomycin injected 2 h after progesterone, on protein synthesis	29
2. Progesterone-facilitated female sexual behavior 2 h after anisomycin injection	31
3. Effect of RU 486 on mean lordosis ratings	38
4. Effect of RU 486 on mean lordosis quotients	40
5. Effect of RU 486 on mean solicitations per minute	42
6. Nuclear progestin receptor concentrations measured in mediobasal hypothalamus-preoptic area and anterior pituitary	47
7. Effect of RU 486 on the concentration of specifically bound [³ H]R 5020 in nuclear extract of mediobasal hypothalamus-preoptic area and anterior pituitary	51
8. Comparison of behavior scores and receptors levels in similarly-treated animals	63

CHAPTER I

INTRODUCTION

During the estrous cycle of rats and guinea pigs, a period of sexual responsiveness is induced by the sequential secretion of ovarian estradiol and progesterone. The concentration of serum estradiol in these animals increases early in proestrus, and reaches its highest level approximately 12 h prior to the occurrence of spontaneous behavioral estrus (Brown-Grant, Exley, and Naftolin, 1970; Nequin, Alvarez, and Schwartz, 1979; Sodersten and Eneroth, 1981). As estradiol levels decrease, serum levels of progesterone increase, and estrous-cycling animals enter a period of sexual receptivity (Feder, Goy, and Resko, 1967; Feder, Resko, and Goy, 1968a; Feder, Resko, and Goy, 1968b) which lasts for approximately 14 h in rats (Blandau, Boling, and Young, 1941) and 8-10 h in guinea pigs (Young et al., 1939). During this period of sexual receptivity, females of both species exhibit lordosis in response to genital stimulation, and female rats exhibit hopping, darting, and rapid ear vibration in response to sexually active males (Edwards and Pfeifle, 1983; Madlafousek and Hlinak, 1977).

Ovariectomized rats (Beach, 1942; Boling and Blandau, 1939) and guinea pigs (Collins et al., 1938; Dempsey,

Hertz, and Young, 1936) can display sexual behavior if the natural sequence of secretion of estradiol and progesterone is reproduced by the exogenous administration of these two hormones. Although it is possible to induce lordosis in ovariectomized rats and guinea pigs 36-48 h after administering estradiol alone, animals treated with estradiol are more likely to exhibit sexual behavior if progesterone is administered a few hours prior to testing (Boling and Blandau, 1939; Dempsey, Hertz, and Young, 1936; Collins et al., 1938). Furthermore, animals which are sexually receptive as a result of the sequential administration of estrogen and progesterone tend to exhibit more soliciting behaviors and more intense lordotic postures than animals which are sexually receptive as a result of estrogen treatment alone (Beach, 1942; Wallen and Thornton, 1979).

In estrogen-primed ovariectomized rats, the period of progesterone-facilitated sexual behavior begins approximately 4 h after subcutaneous progesterone injection (Boling and Blandau, 1939), or 1-2 h after intravenous progesterone injection (Barfield et al., 1983; Glaser, Rubin, and Barfield, 1983; Kubli-Garfias and Whalen, 1977; Meyerson, 1972). However, some investigators reported that estrogen-primed ovariectomized rats exhibited sexual behavior less than 1 h after

progesterone administration; in 30 min if progesterone was injected intravenously (Lisk, 1960; McGinnis et al., 1981b), and in 15 min if progesterone was implanted directly into the brain (Luttge and Hughes, 1976; Ross et al., 1971).

Earlier studies reported that once the period of progesterone-facilitated sexual behavior ended, the administration of a second dose of progesterone would not initiate a second period of sexual receptivity in rats (Blaustein and Wade, 1977; Marrone, Rodriguez-Sierra and Feder, 1977; Nadler, 1970) or guinea pigs (Feder and Marrone, 1977; Morin, 1977; Zucker, 1966). However, later studies revealed that if the supplemental dose of progesterone were large enough, then it might result in lordosis responses (Blaustein, 1982b; Hansen and Sodersten, 1979), suggesting that the animals are merely hyposensitive to progesterone, rather than insensitive.

In a number of studies, estrogen-primed ovariectomized rats and guinea pigs exhibited sexual behavior after cannulae filled with progesterone were implanted directly into specific brain regions. As a result of these studies, different neuroanatomical regions were offered as candidates for the site of progesterone action, including the mediobasal hypothalamus (Morin and Feder, 1974; Powers, 1972), the mesencephalic reticular formation (Ross

et al., 1971; Yanase and Gorski, 1976), the interpeduncular nucleus (Luttge and Hughes, 1976), and the caudate putamen (Yanase and Gorski, 1976). In a more recent study, Rubin and Barfield (1983) reported that progesterone implants facilitated sexual behavior when placed bilaterally in the ventromedial hypothalamus, but not when placed in the mesencephalic reticular formation, the preoptic area, the midbrain central gray area, the caudate-putamen, or the hippocampus. Since a low dose of estrogen was used to prime rats in this study, perhaps implants of progesterone placed in areas outside of the ventromedial hypothalamus did not facilitate sexual behavior because animals did not receive a high enough priming dose of estrogen beforehand.

Relative to other brain regions, the ventromedial hypothalamus contains a large number of estradiol-induced high-affinity progesterone-specific binding sites. Estrogen treatment increases the concentration of cytosol progestin receptors in both the hypothalamus-preoptic area and pituitary gland of ovariectomized rats (Kato and Onouchi, 1977; MacLusky and McEwen, 1978; Moguilewsky and Raynaud, 1979a), but has no effect on receptor levels in the midbrain or cerebral cortex (MacLusky and McEwen, 1978), the amygdala or hippocampus (Moguilewsky and Raynaud, 1979a), or the reticular formation (Kato and

Onouchi, 1977). Parsons et al. (1982a) examined different regions within the hypothalamus-preoptic area of estradiol-treated ovariectomized rats and measured elevated levels of cytosol progesterin receptors in the medial, periventricular, and suprachiasmatic nuclei of the preoptic area, the periventricular anterior hypothalamus, the ventromedial nucleus, and the arcuate-median eminence.

Blaustein and Feder (1979) observed that when ovariectomized guinea pigs were injected with a behaviorally effective dose of estradiol, the concentration of cytosol progesterin receptors increased in the hypothalamus, the preoptic area-septum and the pituitary gland. Although the same dose of estradiol resulted in a small increase in the level of progesterin receptors in the central midbrain sample, no detectable change was observed in the cerebral cortex, amygdala or cerebellum. More recently, Thornton et al. (1986) measured the level of cytosol progesterin receptors induced by estradiol benzoate treatment in microdissected areas of the hypothalamus-preoptic area of ovariectomized guinea pigs. Estradiol benzoate caused a significant induction over baseline levels in the arcuate-median eminence, the periventricular area, the medial preoptic area, the ventromedial hypothalamic nucleus, and the anterior hypothalamic nucleus. No increase in cytosol progesterin

receptors was measured in the medial amygdala or the dorsomedial hypothalamic nucleus.

Estrogen-induced increases in the concentration of hypothalamic cytosol progestin receptors in ovariectomized rats and guinea pigs, occur during the same time period in which progesterone treatment is most likely to induce sexual behavior. Blaustein and Feder (1979) administered a lordosis-facilitating dose of estradiol to ovariectomized guinea pigs and measured an increase in the concentration of cytosol progestin receptors in the hypothalamus and preoptic area beginning at 24 h and ending 64-88 h after hormone injection. This increase in the concentration of cytosol progestin receptors occurred during the same time period in which the administration of progesterone was more apt to induce a behavioral response in like-treated guinea pigs (Feder et al., 1977). Moguilewsky and Raynaud (1979b) and Parsons et al. (1980) observed that in rats there is also a correlation between hypothalamic cytosol progestin receptor levels and the tendency of progesterone injection to induce sexual behavior. Elevated levels of cytosol progestin receptors were measured in the hypothalami of ovariectomized rats approximately 20 h after estradiol treatment, and continued to be measured until approximately 72 h after the hormone was administered. Progesterone injection most

effectively induced sexual responsiveness in these animals when administered 24-60 h after estradiol treatment.

According to the model generally used to explain how progesterone facilitates sexual behavior after estrogen priming, cytosol progestin receptors are translocated to hypothalamic cell nuclei after being occupied by progesterone. A behaviorally-effective dose of progesterone decreases the concentration of cytosol progestin receptors and increases the concentration of nuclear progestin receptors in the hypothalamus of estrogen-primed ovariectomized guinea pigs (Blaustein and Feder, 1980), as well as rats (Rainbow et al., 1982b). In the study by Blaustein and Feder (1980), ovariectomized guinea pigs received subcutaneous injection of progesterone 40 h after estradiol. The concentration of hypothalamic nuclear progestin receptors increased within 2 h of progesterone treatment, and returned to baseline concentrations 12-24 h after progesterone treatment. Guinea pigs that received a similar hormonal treatment tended to display lordosis in response to manual stimulation at 4 h, but not at 24 h after progesterone treatment (Blaustein, 1982a). A similar correlation was observed in ovariectomized rats which received intravenous injections of progesterone 72 h after receiving subcutaneous implants of Silastic capsules containing

estradiol. McGinnis et al. (1981b) reported that as a result of this hormonal treatment, the concentration of nuclear progestin receptors measured in the mediobasal hypothalami of ovariectomized rats was above baseline levels at 15 min after progesterone injection, while sexual behavior was first observed at 30 min after progesterone injection. This correlation is one of the facts which led to the hypothesis that progesterone facilitates sexual behavior by occupying hypothalamic progestin receptors and inducing the accumulation of these receptors in the nucleus of the cell.

In light of recent reports, it is unclear whether unoccupied progestin receptors are found primarily in the cytosol or the nucleus of target cells. Autoradiographic studies by Sheridan et al. (1979) suggest that unoccupied steroid receptors are in equilibrium between the cytosol and the nucleus. Studies using either cell enucleation (Welshons, Krummel, and Gorski, 1985; Welshons, Liebermann, and Gorski, 1984) or immunocytochemistry (Gasc et al., 1984; King and Greene, 1984) report that steroid receptors are found primarily in the cell nucleus. These authors suggest that as a result of tissue homogenization, receptors that are only loosely associated with the cell nucleus are released into the cytosol fraction. If this were the case, then the cytosol receptors referred to in

this thesis may represent loosely associated, unoccupied receptors which are released from the cell nuclei during tissue homogenization, whereas the nuclear receptors referred to in this thesis may represent tightly associated, occupied receptors. Regardless of the primary cellular location of unoccupied progesterin receptors, receptors occupied with progesterone appear to bind to the nucleus.

In the cell nucleus, receptors occupied with progesterone might induce sexual behavior in rats and guinea pigs by initiating the synthesis of behaviorally-relevant proteins. Rainbow, Davis, and McEwen (1980) reported that subcutaneous injections of the protein synthesis inhibitor, anisomycin, effectively terminated progesterone-facilitated sexual behavior in estrogen-primed ovariectomized rats if administered either 15 min before or up to 5 h after progesterone treatment. It is unlikely that animals failed to show lordosis due to incapacitation in this study, because the same dose of anisomycin did not block sexual behavior induced by long-term estrogen treatment. The authors also reported that the same dose of anisomycin decreased the incorporation of [^3H]leucine into protein by 90% after 30 min and by 80% after 2 h in the medial basal hypothalamus, preoptic area, and corticomедial amygdala of ovariectomized rats.

In a later study, Rainbow et al. (1982a) revealed that cannulae filled with anisomycin inhibited progesterone-facilitated sexual behavior in estrogen-primed ovariectomized rats when implanted directly into the medial basal hypothalamus 15-30 min before progesterone injection. These authors also reported that anisomycin implants in the ventromedial nucleus of the hypothalamus blocked sexual behavior, and inhibited protein synthesis in the medial basal hypothalamus without influencing protein synthesis in the preoptic area, corticomедial amygdala, or pituitary gland. In a similar study, Glaser and Barfield (1984) found that anisomycin-filled cannulas blocked sexual behavior when implanted into the ventromedial hypothalamus, but not when implanted into the preoptic area of midbrain in the region of the interpeduncular nucleus. These studies suggest that progesterone-facilitated sexual behavior occurs in rats and guinea pigs as a result of hypothalamic protein synthesis, which is induced by elevated concentrations of nuclear progestin receptors in the mediobasal hypothalamus.

Progesterone-facilitated sexual behavior in estrogen-primed ovariectomized rats and guinea pigs is also blocked by the administration of RU 486. RU 486 is a synthetic progesterone antagonist which binds, in vitro, with a high

affinity for cytosol progestin receptors from guinea pig brain (Brown and Blaustein, 1984a), rat brain (Brown and Blaustein, unpublished data; Etgen and Barfield, 1986), and mammalian uteri (Gravanis et al., 1985; Philibert et al., 1982; Rauch et al., 1985). Although RU 486 has a greater affinity than progesterone for intracellular progestin receptors, RU 486-bound receptors appear to be functionally inactive in the nucleus (Baulieu, 1985; Rabe, Kiesel, and Runnebaum, 1985; Spilman et al., 1986). The administration of RU 486 at 1 h before progesterone treatment blocks sexual receptivity in estrogen-primed ovariectomized guinea pigs (Brown and Blaustein, 1984a) and rats (Etgen and Barfield, 1986), suggesting that the progesterone antagonist inhibits sexual behavior by interacting with progestin receptors. This same conclusion was reached in a recent study by Blaustein, Finkbohner, and Delville (1987), in which sexual behavior induced by estradiol treatment alone was not blocked by the administration of RU 486 in ovariectomized rats.

Not only does it appear that sexual responsiveness in rats and guinea pigs is induced by the accumulation of occupied progestin receptors in hypothalamic cell nuclei, it also appears that the period of sexual behavior terminates as a result of the loss of progestin receptors from hypothalamic cell nuclei. Brown and Blaustein (1985)

implanted Silastic capsules containing progesterone into ovariectomized guinea pigs 40 h after estradiol priming and observed that, by 18 h after capsule insertion, none of the animals continued to exhibit sexual receptivity. At 18 h after progesterone treatment, the concentration of hypothalamic nuclear progestin receptors for similarly-treated animals returned to baseline levels after being elevated for 15 h.

In related studies, the period of sexual receptivity was extended by hormonal treatments which maintained elevated concentrations of progesterone-occupied receptors in hypothalamic cell nuclei. Brown and Blaustein (1984c) extended the duration of sexual receptivity in estrogen-primed ovariectomized guinea pigs by administering a second injection of progesterone 8 h after the first. At 14 h after the initial progesterone injection, the concentration of hypothalamic nuclear progestin receptors was still above baseline levels in the recipients of supplemental progesterone, and 80% of the animals which received supplemental progesterone continued to display sexual behavior. In animals which did not receive supplemental progesterone, the period of sexual receptivity ended 2 h earlier, and the concentration of hypothalamic nuclear progestin receptors returned to baseline level 10-14 h after the initial dose of

progesterone. The administration of supplemental estradiol concurrent with progesterone also maintains elevated levels of hypothalamic nuclear progestin receptors (Blaustein, 1982a) and extends the period of progesterone-facilitated sexual behavior in estrogen-primed ovariectomized rodents (Joslyn and Feder, 1971; Shivers et al., 1980).

On the other hand, the period of sexual receptivity is shortened by hormonal treatments which lower the concentration of progesterone-occupied receptors in hypothalamic cell nuclei. Brown and Blaustein (1986) injected RU 486 at 4 h after progesterone treatment in estrogen-primed ovariectomized guinea pigs and observed that the period of progesterone-facilitated sexual behavior ended 3 h earlier. RU 486 treatment also decreased the level of progesterone-occupied nuclear progestin receptors in the mediobasal hypothalamus of animals killed at either 6 and 10 h after progesterone injection. The RU 486-induced abbreviation of guinea pig sexual behavior was overcome by administration of a large dose of progesterone, but not by a large dose of cortisol. This suggests that in spite of being a potent glucocorticoid antagonist (Gagne, Pons, and Philibert, 1985; Moguilewsky and Philibert, 1984), RU 486 inhibits sexual receptivity as a result of actions upon the

progestin system rather than the glucocorticoid system. This evidence supports the hypothesis that the period of sexual behavior in rats and guinea pigs terminates in conjunction with a decrease in the concentration of progesterone-occupied receptors in hypothalamic cell nuclei, and that the duration of sexual receptivity in these animals can be modified through the administration of hormones which either sustain or abate elevated concentrations of hypothalamic nuclear progestin receptors.

While in guinea pigs there is close concordance between the decrease in the concentration of occupied nuclear progestin receptors and the termination of sexual behavior, the same does not appear to be true for rats. Two studies examined the retention of nuclear progestin receptors in the mediobasal hypothalamus and preoptic area of ovariectomized, estrogen-primed rats after a behaviorally effective injection of progesterone. In the first study, McGinnis et al. (1981b) reported that rats displayed sexual behavior as late as 10 h after an intravenous progesterone injection, even though the concentration of nuclear progestin receptors had returned to baseline levels by that time. In the second study, by Ahdieh et al. (1985), nuclear progestin receptor concentrations returned to baseline levels by 8 h after a

subcutaneous injection of progesterone, while animals continued to display sexual behavior some 8 h later. In both of these studies, the concentration of hypothalamic nuclear progestin receptors returned to baseline levels approximately 10 h before the loss of sexual responsiveness.

The rats studied by McGinnis et al. (1981b) and Ahdieh et al. (1985) exhibited a long delay between the loss of hypothalamic nuclear progestin receptors and the termination of female sexual behavior. Since a similar delay is not observed in guinea pigs, perhaps factors which have a longer lifespan in rats than in guinea pigs maintain sexual behavior after receptor concentrations return to baseline. Another possibility is that in rats, but not in guinea pigs, even a low concentration of progesterone-occupied receptors in hypothalamic cell nuclei will maintain sexual receptivity. The hypothesis of this thesis is that the initial increase of progestin receptors observed in the hypothalamic cell nuclear fraction may initiate cellular events that maintain the period of sexual receptivity for hours after nuclear progestin receptor levels return to baseline. To test this hypothesis, this thesis examined the role of continued hypothalamic protein synthesis and elevated levels of nuclear-bound hypothalamic progestin receptors

on maintenance of the period of sexual behavior in ovariectomized estrogen-primed rats treated with progesterone.

C H A P T E R I I

METHODS

Subjects

Female rats (Charles River Breeding Laboratories, Wilmington, MA) were caged in groups of four and ovariectomized no less than seven days after arrival. Food (Rodent Laboratory Chow 5001, Ralston Purina, St. Louis, MO) and tap water were available ad libitum. Room lights were on from 2300 to 1300 h, and room temperature was approximately 23°C. Animals with a body weight of 145-175 g were ovariectomized via a single, midventral incision under methohexital sodium anesthesia (52 mg/kg body weight, Brevital Sodium, Eli Lilly, Indianapolis, IN).

Unless otherwise stated, each animal was injected s.c. with 2 µg of 17β-estradiol benzoate dissolved in 0.1 ml of sesame oil, one and one-half weeks after ovariectomy. Forty-four hours later, rats were injected s.c. with either oil vehicle or progesterone dissolved in 0.1 ml of sesame oil containing 5% benzyl alcohol and 15% benzyl benzoate. Anisomycin was dissolved in 0.9% saline (100 mg/kg body weight, pH 7.0) and was injected via three s.c. injections: one in each axillary region and one in the

nape of the neck. RU 486 (17β -hydroxy- 11α -[4-dimethylaminophenyl]- 17β -[1-propynyl]-estra-4,9-diene-3-one, Roussel-Uclaf, Romainville, France) was dissolved in the same sesame oil vehicle as progesterone and was injected via two lateral 0.2-ml s.c. injections, one on each side.

Tests for sexual receptivity

Each animal was tested for sexual behavior in circular Plexiglass arenas, 53 cm across by 41 cm high, with pine shavings covering the floor. All testing was done during the dark phase of the lighting cycle under either dim white light or direct red light illumination. The test consisted of ten vigorous mounts by either of two sexually-experienced males already present in the arena. The intensity of the lordosis response was scored on a scale of 1 to 3 in a modification of the method used by Hardy and Debold (1971). The lordosis rating was defined as the mean score for ten mounts. The lordosis quotient was defined as (number of lordoses/number of mounts) \times 100. Solicitations per minute was defined as the total observations of ear-wiggling, hopping or darting divided by the length of the testing period in minutes.

Brain dissection

Tissue samples from each animal consisted of either the anterior pituitary, the pooled mediobasal hypothalamus and preoptic area, or a slice of cortex. Early in the dark phase of their lighting cycle, rats were killed by decapitation, and their brains and pituitary glands were rapidly removed. The anterior pituitary was dissected from the rest of the pituitary. The mediobasal hypothalamus and preoptic area were dissected on a chilled block in the manner described by Brown and Blaustein (1984b). The wedge-shaped mediobasal hypothalamus sample was bounded rostrally by the caudal edge of the optic chiasm and caudally by the caudal edge of the mammillary bodies. Cuts were made extending from the lateral hypothalamic fissure to the midpoint of the corpus callosum to form the lateral boundaries, and a cut was made through the level of the fornix to form the dorsal boundary. The preoptic area sample extended 2 mm rostrally from the hypothalamic section and included the anterior hypothalamus and preoptic area. A line continuous with the lateral ventricles formed the lateral boundaries, and the anterior commissure formed the dorsal boundary.

Protein synthesis assay

Protein synthesis was measured using the methods of Rainbow, Davis, and McEwen (1980). Rats were injected i.p. with 25 μ Ci of [3 H]leucine (New England Nuclear, Boston, MA) dissolved in 0.2 ml of saline. Fifteen minutes later, each rat was decapitated, and its mediobasal hypothalamus and a piece of overlaying cortex of equivalent mass were dissected and placed individually into polycarbonate tubes containing 2 ml of cold water. Each sample was homogenized for 5 sec at low speed with a Brinkmann Polytron tissue grinder. Next, 200 μ l of 60% TCA was added to each sample, which then was vortexed and put on ice. After chilling for 30 min, the samples were centrifuged at 48,000 x g for 10 min. In order to determine the concentration of [3 H]leucine in the TCA soluble fraction, 1 ml samples of supernatant were transferred to scintillation vials, scintillation fluid was added, and the samples were counted in a Packard Tri-Carb 300C scintillation counter. The remaining supernatants were discarded, and the pellets were resuspended twice in 2 ml of 6% TCA followed by centrifugations at 48,000 x g for 10 min. The pellets were then dissolved overnight in 500 μ l 1 M NaOH. In order to determine the concentration of [3 H]leucine in the

TCA insoluble fraction, 500 μ l of 1 M HCl was added to each sample, samples were transferred into scintillation vials, scintillation fluid was added and the samples were counted. The rate of [3 H]leucine incorporation was expressed as dpm in the TCA insoluble fraction/dpm in the TCA soluble fraction.

Nuclear progestin receptor assay

Cell nuclei were isolated using a modification of the method by Kranzler et al. (1984). All procedures were conducted at 0-4°C. Tissue samples from two similarly-treated rats were pooled in 1.0 ml of Buffer A (0.32 M sucrose, 1 mM KH_2PO_4 , 1 mM MgCl_2 , 10% glycerol, 12 mM monothioglycerol, pH 7.2) and homogenized in a glass tissue grinder with a Teflon pestle. The homogenates were transferred to polycarbonate tubes and the tissue grinders were rinsed with 500 μ l of Buffer A. After centrifugation at 1000 x g for 5 min, the supernatants were decanted, and the pellets were dispersed in 350 μ l of Buffer A. The sucrose concentration was brought up to 1.0 M by adding 400 μ l of Buffer C (1.6 M sucrose, 1 mM KH_2PO_4 , 1 mM MgCl_2 , 10% glycerol, pH 7.2) and vortexing. Seven-hundred-fifty microliters of Buffer C was slowly added while tipping the polycarbonate tube so as to avoid mixing

the two sucrose densities. After centrifugation at 27,000 x g for 20 min, the supernatants were decanted and the walls of the polycarbonate tubes dried.

Nuclear progesterin receptors were assayed using a modification of the method of Blaustein and Feder (1980). The nuclear pellets were dispersed in 285 μ l of Buffer TEGT (10 mM Tris-HCl, 1.5 mM Na₂EDTA, 10% glycerol, 12 mM monothioglycerol, pH 7.4). After the samples incubated for 15 min, 285 μ l of Buffer TEGTK_{1.0} (TEGT containing 1.0 M KCl) were added to each tube, which was vortexed for 10 sec and allowed to incubate for 15-60 min, with occasional vortexing. After this incubation period, the tubes were centrifuged at 48,000 x g for 5 min and the supernatants were decanted. DNA content of the nuclear pellets was determined by the method of Burton (1956).

Two-hundred-fifty microliter samples of the nuclear extract supernatant were added to 12 x 75 mm glass culture tubes containing a final concentration (Reading and Blaustein, 1984) of 1.5 nM of [³H]R 5020 (New England Nuclear, Boston, MA; spec. act. = 87.0 Ci/mmol) dissolved in 47 μ l of Buffer TEGTK_{0.5} (TEGT + 0.5 M KCl, pH 7.4) and 3 μ l of ethanol with or without a 250-fold excess concentration of progesterone. After an overnight incubation, 250 μ l aliquots were applied to 4.5 x 68-mm Sephadex LH-20 (Pharmacia Fine Chemicals, Picataway, NJ)

columns that had been previously equilibrated with Buffer TEGTK_{0.5}. After the samples descended into the columns, 150 μ l of Buffer TEGTK_{0.5} were added onto each column. Twenty minutes after each sample application, the macromolecular fraction was eluted into scintillation vials with 700 μ l of Buffer TEGTK_{0.5}. Five milliliters of toluene-based scintillation fluid containing 33% Triton X-100 was added to each vial, and the radioactivity was counted using a Packard Tri-Carb model 300C spectrophotometer (Packard, Downer Grove, IL). Results are expressed as femtomoles of [³H]R 5020 specifically bound per mg DNA.

Statistical analysis

Data were analyzed by two-way analysis of variance, one-way analysis of variance, or Student's t test, as appropriate. Results were considered statistically significant if $p < 0.05$.

C H A P T E R I I I

EXPERIMENT 1: INHIBITING PROGESTERONE- FACILITATED SEXUAL BEHAVIOR WITH ANISOMYCIN

Rationale

It has been shown that the retention of hypothalamic nuclear progestin receptors is required for progesterone-facilitated sexual behavior in guinea pigs. After progesterone injection, the concentration of hypothalamic nuclear progestin receptors rises and remains elevated until the period of sexual behavior terminates (Blaustein, 1982a; Brown and Blaustein, 1985). In rats, unlike guinea pigs, the concentration of hypothalamic nuclear progestin receptors declines to baseline levels within 8 h of progesterone injection, even though sexual behavior is seen for a subsequent 10 h (Ahdieh et al., 1986; McGinnis et al., 1981).

It has been suggested that progesterone-facilitated sexual behavior in ovariectomized estrogen-primed rats is dependent on protein synthesis. The protein synthesis inhibitor, anisomycin, has been shown to block progesterone-facilitated sexual behavior if injected 15 min prior to or up to 4 h after insertion of estrogen capsules (Parsons et al., 1982b; Rainbow, Davis, and

McEwen, 1980) or if injected 15 min prior to or up to 5 h after progesterone treatment (Rainbow, Davis, and McEwen, 1980). It has been shown that sexual behavior is inhibited even when anisomycin is implanted intracerebrally directly into the ventromedial nucleus of the hypothalamus (Glaser and Barfield, 1984; Rainbow et al., 1982a). The purpose of this experiment was to determine if sexual behavior in rats is dependent on ongoing protein synthesis even as late as 10 h after progesterone injection. To test this, anisomycin was injected in ovariectomized estrogen-primed rats at various times after the injection of progesterone. We first determined the dose of anisomycin necessary to suppress protein synthesis in the mediobasal hypothalamus as measured by [^3H]leucine incorporation into TCA-precipitable protein.

Procedure

In the first part of this experiment, ovariectomized rats were injected with 1 mg of progesterone 44 h after receiving 2 μg of estradiol benzoate. Two hours after progesterone treatment, rats were injected with either saline vehicle or one of two doses of anisomycin (100 or 150 mg anisomycin/kg body weight). Two hours after the

administration of anisomycin or saline, each rat was injected i.p. with 25 μ Ci of [3 H]leucine and killed fifteen minutes later. Mediobasal hypothalamus and a piece of overlying cortex of equivalent mass were assayed for incorporation of [3 H]leucine into protein.

In the second part of this experiment, ovariectomized rats were injected with 500 μ g of progesterone 44 h after receiving 2 μ g of estradiol benzoate. At 2 or 10 h after progesterone treatment, rats were injected with either 100 mg of anisomycin/kg body weight (EB-P-Ani) or saline vehicle (EB-P-Veh). In order to determine the extent of sexual behavior induced by estradiol in the absence of progesterone, the study included a control group which received estradiol benzoate followed by vehicles (EB-Veh-Veh).

Two hours after the injection of either anisomycin or saline, rats were tested for sexual behavior and the mean scores for the anisomycin-injected animals (EB-P-Ani) were compared to the mean scores of oil-injected animals (EB-P-Veh).

Results

Protein synthesis

Anisomycin significantly inhibited protein synthesis in the mediobasal hypothalamus when injected in either the low dose ($t_6=5.904$, $p<0.002$; Fig. 1) or high dose ($t_6=7.675$, $p<0.001$). The higher dose caused significantly more inhibition of protein synthesis than the lower dose of anisomycin (87% vs. 70%; $t_6=4.088$, $p<0.01$).

Sexual behavior - anisomycin injected 2 h after progesterone

Anisomycin caused a significant decrease in lordosis quotients ($t_{10}=3.468$, $p<0.01$; Fig. 2, left panel), lordosis ratings ($t_{10}=5.757$, $p<0.001$), and solicitations per minute ($t_{10}=3.490$, $p<0.01$).

Sexual behavior - anisomycin injected 10 h after progesterone

Anisomycin caused a significant decrease in lordosis quotients ($t_{18}=2.191$, $p<0.05$; Fig. 2, right panel) and lordosis ratings ($t_{18}=2.644$, $p<0.02$), but not in solicitations per minute ($t_{18}=1.571$, $p>0.1$).

Figure 1

Effect of anisomycin injected 2 h after progesterone, on protein synthesis. Error bars represent one standard error. Ovariectomized rats were injected with 1 mg of progesterone 44 h after 2 µg of estradiol benzoate. Two hours after progesterone treatment, animals were injected with either 100 or 150 mg of anisomycin/kg or saline vehicle. Two hours after anisomycin or saline injection, animals were injected with 25 µCi of [³H]leucine. Animals were killed fifteen minutes later and mediobasal hypothalamus (MBH) and cortex (CRT) were assayed for incorporation of [³H]leucine into protein. Rate of [³H]leucine incorporation is expressed as (dpm in TCA insoluble fraction/dpm in TCA soluble fraction), n = 4 per group.

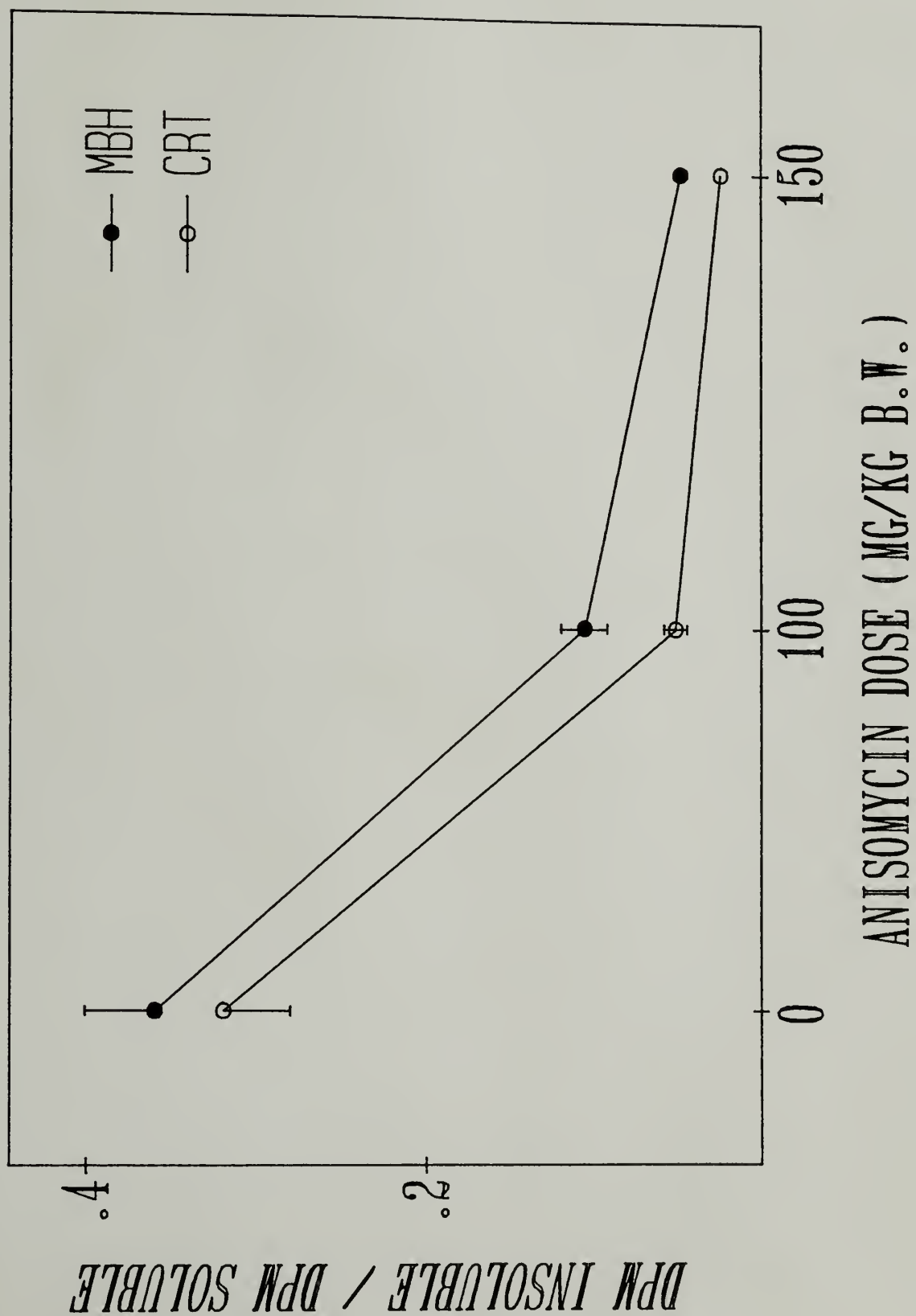
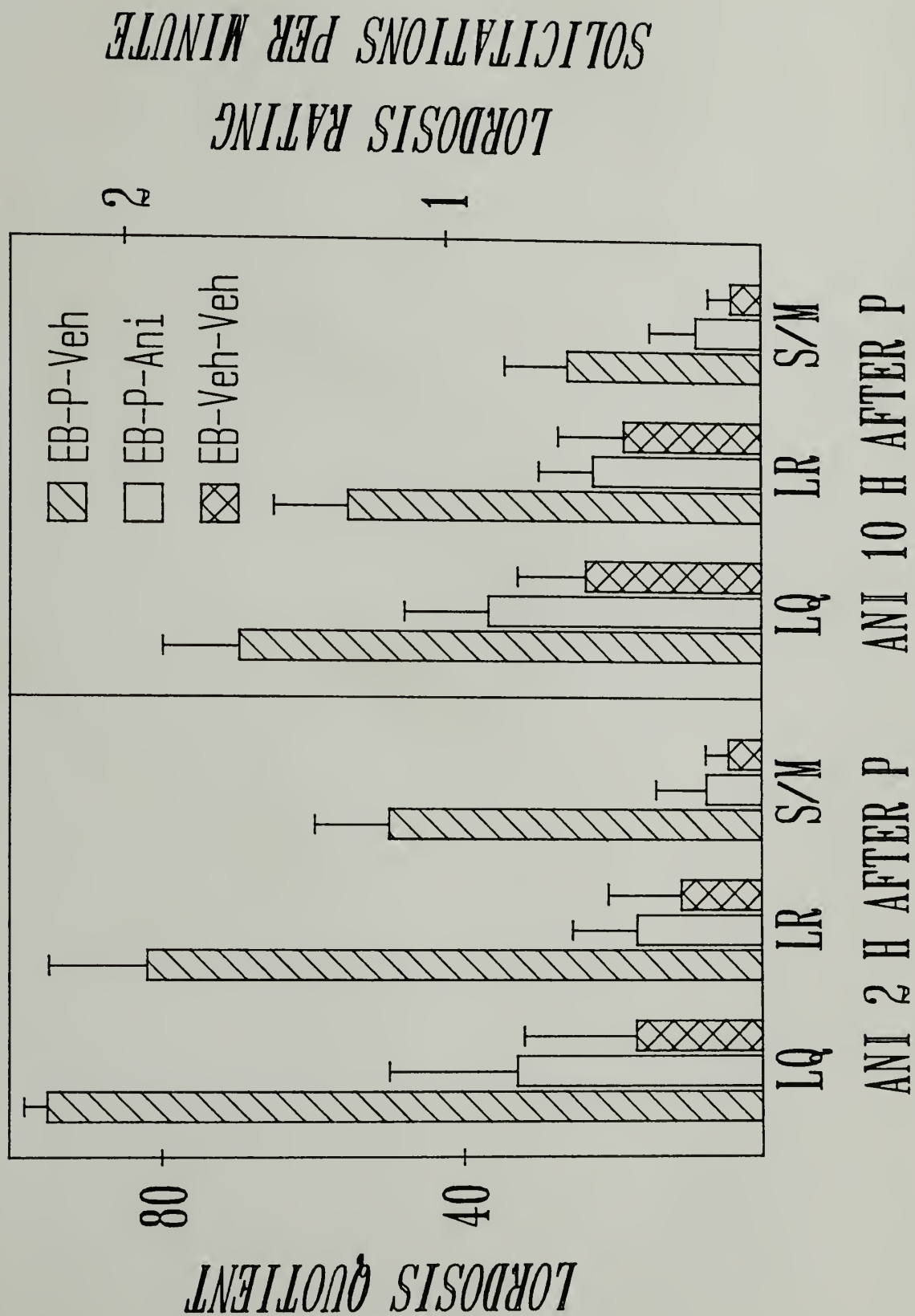


Figure 2

Progesterone-facilitated female sexual behavior 2 h after anisomycin injection. Error bars represent one standard error. Ovariectomized rats were injected with 500 μ g of progesterone 44 h after 2 μ g of estradiol benzoate.

Anisomycin (EB-P-Ani) or vehicle (EB-P-Veh) was administered either 2 h (left panel, n = 6 per group) or 10 h (right panel, n = 10 per group) after progesterone injection, and rats were tested for sexual behavior 2 h later. A control group received estradiol benzoate followed by oil vehicle at 44 h and then saline vehicle either 2 or 10 h later (EB-Veh-Veh).



C H A P T E R I V

EXPERIMENT 2: INHIBITING PROGESTERONE-
FACILITATED SEXUAL BEHAVIOR WITH RU 486

Rationale

In estrogen-primed ovariectomized rats, the concentration of hypothalamic nuclear progestin receptors returns to baseline levels by 8 h after progesterone injection, while sexual behavior lasts until approximately 18 h after progesterone injection (Ahdieh et al., 1986; McGinnis et al., 1981). The progesterone antagonist, RU 486, has been shown to inhibit progesterone-facilitated sexual behavior in estrogen-primed ovariectomized rats if administered up to 1 h after progesterone treatment (Etgen and Barfield, 1986) and in guinea pigs if injected as late as 4 h after progesterone treatment (Brown and Blaustein, 1984a; Brown and Blaustein, 1986). The purpose of this experiment was to determine how late after progesterone-injection RU 486 could inhibit sexual behavior in ovariectomized estrogen-primed rats.

If RU 486 inhibits progesterone's action by competing for intracellullar progestin receptors (Philibert et al., 1982), then injecting RU 486 prior to the time that nuclear progestin receptors have returned to baseline

concentrations should inhibit sexual behavior. Conversely, injection of the antagonist after nuclear progesterin receptors have returned to baseline concentrations should not inhibit sexual behavior. Therefore, based on the previous nuclear progesterin receptor profile, it was expected that RU 486 injected either 2 or 6 h after progesterone treatment would lead to an early termination of the period of sexual behavior, while RU 486 injected 10 h after progesterone treatment would have no effect on the duration of sexual responsiveness.

Procedure

Ovariectomized rats were injected with 500 μ g of progesterone 44 h after receiving 2 μ g of estradiol benzoate. At 2, 6, or 10 h after progesterone treatment, rats were injected with either 5 mg of RU 486 (EB-P-RU) or oil vehicle (EB-P-Veh). In order to determine the extent of sexual behavior induced by estradiol in the absence of progesterone, the study also included a control group which received estradiol benzoate followed by vehicle (EB-Veh-Veh).

At 4, 8, and in some cases, 12 h after the injection of RU 486 or oil, rats were tested for sexual behavior and

the mean scores of RU 486-injected animals (EB-P-RU) were compared to the mean scores of oil-injected animals (EB-P-Veh).

Results

RU 486 injected 2 h after progesterone

Two way analysis of variance with repeated measures revealed that RU 486 caused a significant decrease in lordosis ratings ($F_{1,11}=11.800$, $p<0.01$; Fig. 3), lordosis quotients ($F_{1,11}=12.546$, $p<0.005$; Fig. 4), and solicitations per minute ($F_{1,11}=8.192$, $p<0.025$; Fig. 5).

At 4 h after treatment with either RU 486 or oil, these two groups showed no significant difference in lordosis ratings ($t_{11}=1.501$, $p>0.1$), lordosis quotients ($t_{11}=1.676$, $p>0.1$), or solicitations per minute ($t_{11}=1.305$, $p>0.2$). At 8 h after treatment, RU 486-injected animals displayed significantly lower lordosis ratings ($t_{11}=6.067$, $p<0.001$), lordosis quotients ($t_{11}=6.314$, $p<0.001$), and solicitations per minute ($t_{11}=2.979$, $p<0.02$). At 12 h after treatment, RU 486-injected animals displayed a trend towards lower lordosis ratings ($t_{11}=1.916$, $p<0.1$) and lordosis quotients ($t_{11}=2.032$, $p<0.1$); solicitations per minute were not significantly different ($t_{11}=1.329$, $p>0.2$).

RU 486 injected 6 h after progesterone

Two way analysis of variance with repeated measures revealed that RU 486 caused a significant decrease in lordosis ratings ($F_{1,19}=8.436$, $p<0.01$), lordosis quotients ($F_{1,19}=7.532$, $p<0.025$), and solicitations per minute ($F_{1,19}=6.458$, $p<0.025$).

At 4 h after treatment with either RU 486 or oil, RU 486-injected animals displayed a trend towards lower lordosis ratings ($t_{19}=1.765$, $p<0.1$), lordosis quotients ($t_{19}=1.951$, $p<0.1$), and solicitations per minute ($t_{19}=2.029$, $p<0.1$). At 8 h after treatment, RU 486-injected animals displayed significantly lower lordosis ratings ($t_{19}=3.557$, $p<0.01$), lordosis quotients ($t_{19}=3.311$, $p<0.01$), and solicitations per minute ($t_{19}=2.408$, $p<0.05$). At 12 h after treatment, there was no significant difference in lordosis ratings ($t_{19}=1.139$, $p>0.2$), lordosis quotients ($t_{19}=0.869$, $p>0.2$), or solicitations per minute ($t_{19}=1.626$, $p>0.1$).

RU 486 injected 10 h after progesterone

Two way analysis of variance with repeated measures revealed that RU 486 caused a significant decrease in lordosis ratings ($F_{1,15}=11.842$, $p<0.005$) and lordosis quotients ($F_{1,15}=17.337$, $p<0.001$), but not in solicitations per minute ($F_{1,15}=1.610$, $p>0.2$).

At 4 h after treatment with either RU 486 or oil, RU 486-injected animals displayed significantly lower lordosis ratings ($t_{15}=2.428$, $p<0.05$) and lordosis quotients ($t_{15}=2.838$, $p<0.02$). At 8 h after treatment, RU 486-injected animals continued to display significantly lower lordosis ratings ($t_{15}=2.714$, $p<0.02$) and lordosis quotients ($t_{15}=2.853$, $p<0.02$). Solicitations per minute were not significantly different at either 4 h ($t_{15}=1.132$, $p>0.2$) or 8 h ($t_{15}=1.506$, $p>0.1$) after treatment with RU 486.

Figure 3

Effect of RU 486 on mean (\pm S.E.M.) lordosis ratings. Ovariectomized rats were injected with 500 μ g of progesterone or oil vehicle 44 h after 2 μ g of estradiol benzoate. Rats received 5 mg of RU 486 (EB-P-RU) or oil vehicle (EB-P-Veh) either 2 h (top panel, n = 6-7 per group), 6 h (middle panel, n = 10-11 per group), or 10 h (bottom panel, n = 8-9 per group) after progesterone treatment and were tested for sexual behavior 4, 8, and in some cases, 12 h later. A control group received estradiol benzoate followed by oil vehicles (EB-Veh-Veh).

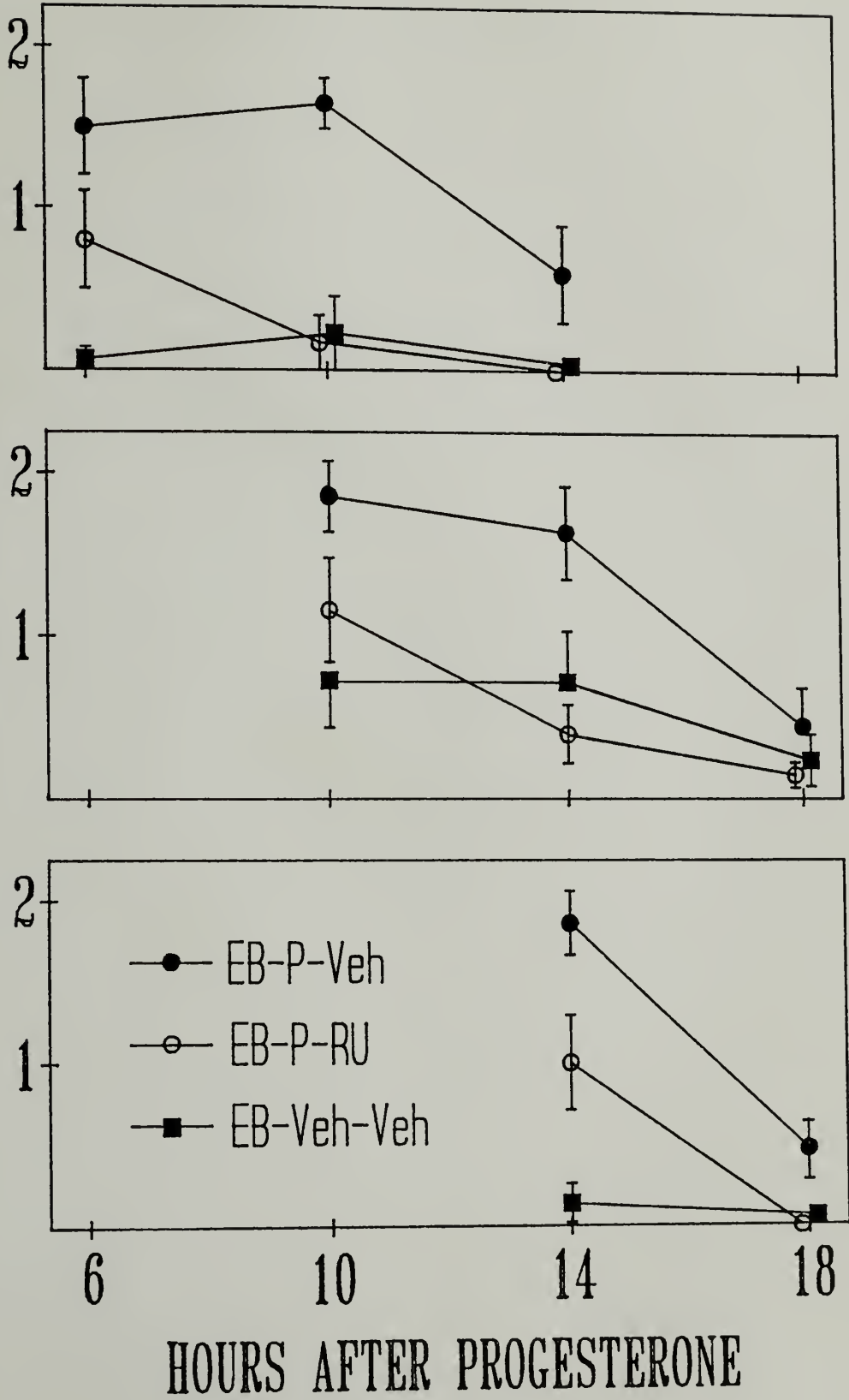
LORDOSIS RATING

Figure 4

Effect of RU 486 on mean (\pm S.E.M.) lordosis quotients. Ovariectomized rats were injected with 500 μ g of progesterone or oil vehicle 44 h after 2 μ g of estradiol benzoate. Rats received 5 mg of RU 486 (EB-P-RU) or oil vehicle (EB-P-Veh) either 2 h (top panel, n = 6-7 per group), 6 h (middle panel, n = 10-11 per group), or 10 h (bottom panel, n = 8-9 per group) after progesterone treatment and were tested for sexual behavior 4, 8, and in some cases, 12 h later. A control group received estradiol benzoate followed by oil vehicles (EB-Veh-Veh).

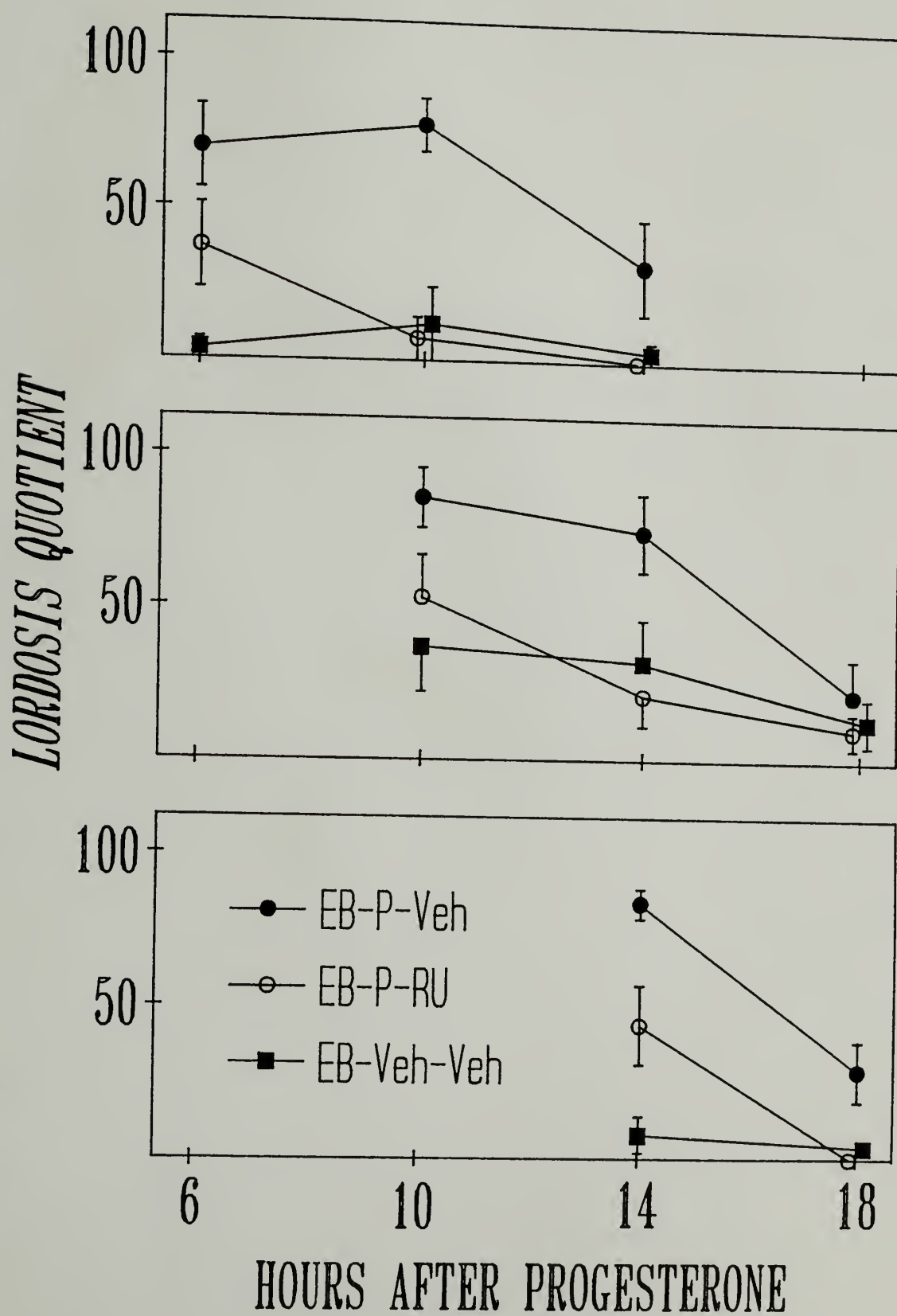
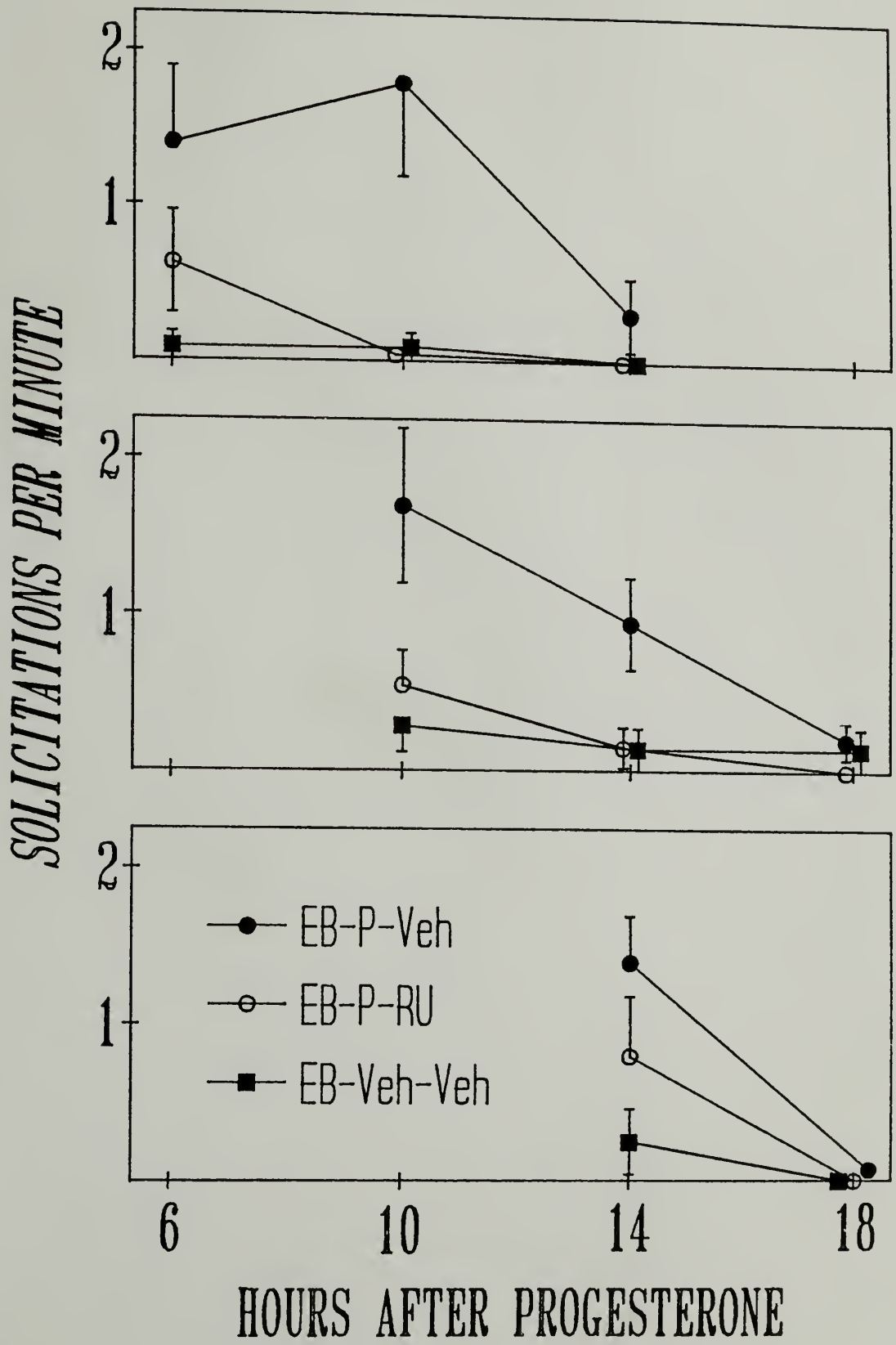


Figure 5

Effect of RU 486 on mean (\pm S.E.M.) solicitations per minute. Ovariectomized rats were injected with 500 μ g of progesterone or oil vehicle 44 h after 2 μ g of estradiol benzoate. Rats received 5 mg of RU 486 (EB-P-RU) or oil vehicle (EB-P-Veh) either 2 h (top panel, n = 6-7 per group), 6 h (middle panel, n = 10-11 per group), or 10 h (bottom panel, n = 8-9 per group) after progesterone treatment and were tested for sexual behavior 4, 8, and in some cases, 12 h later. A control group received estradiol benzoate followed by oil vehicles (EB-Veh-Veh).



C H A P T E R V

EXPERIMENT 3: DECREASES IN THE CONCENTRATION
OF NUCLEAR PROGESTIN RECEPTORS OVER TIME

Rationale

In the previous experiment, RU 486 inhibited sexual behavior when injected 10 h after progesterone treatment. This was surprising because the concentration of nuclear progestin receptors was reported to have returned to baseline levels by this time (Ahdieh et al., 1986; McGinnis et al., 1981). One possible explanation for this paradox is that there actually is an elevated level of nuclear progestin receptors at 10 h, but that the receptor assay is not sensitive enough to show a difference between oil-injected and progesterone-injected groups. We attempted to assay the concentration of nuclear progestin receptors at various times after progesterone injection using a modification of the standard nuclear progestin receptor assay (Blaustein, 1982a; Blaustein, 1982b; Blaustein and Feder, 1980). Prior to this, we compared the concentration of nuclear progestin receptors that was obtained using the new technique to the concentration obtained using the old technique.

Procedure

In the first part of this experiment, ovariectomized rats were injected with 2 mg of progesterone or oil vehicle 44 h after receiving 10 μ g of estradiol benzoate. Two hours after injection of either progesterone or oil, rats were killed and mediobasal hypothalamus-preoptic areas from similarly-treated animals were pooled. Cell nuclei were isolated from aliquots of each tissue pool using either the modified technique described in Methods, or the standard technique described previously (Blaustein, 1982a; Blaustein, 1982b; Blaustein and Feder, 1980). Nuclear progestin receptors and DNA contents for all samples were measured as described in Methods.

In the second part of this experiment, ovariectomized rats were injected with 500 μ g of progesterone or oil vehicle 44 h after receiving 2 μ g of estradiol benzoate. At 10, 14, or 18 h after injection of either progesterone or oil, rats were killed and mediobasal hypothalamus-preoptic area and anterior pituitary were removed. Cell nuclei were isolated as described in Methods, and the difference in nuclear progestin receptor concentrations between oil-injected and progesterone-injected animals was determined at the various times mentioned.

Results

Standard vs. modified cell nuclei isolation

Compared to the receptor levels measured in oil-injected animals, the elevated concentration of nuclear progestin receptors measured in progesterone-injected animals did not increase significantly ($t_6=0.842$, $p>0.2$; $n = 4$ per group) when the modified method (32 ± 4 fmoles [^3H]R 5020 bound/mg DNA) was used to isolate cell nuclei instead of the standard method (27 ± 4 fmoles [^3H]R 5020 bound/mg DNA).

Mediobasal hypothalamus and preoptic area

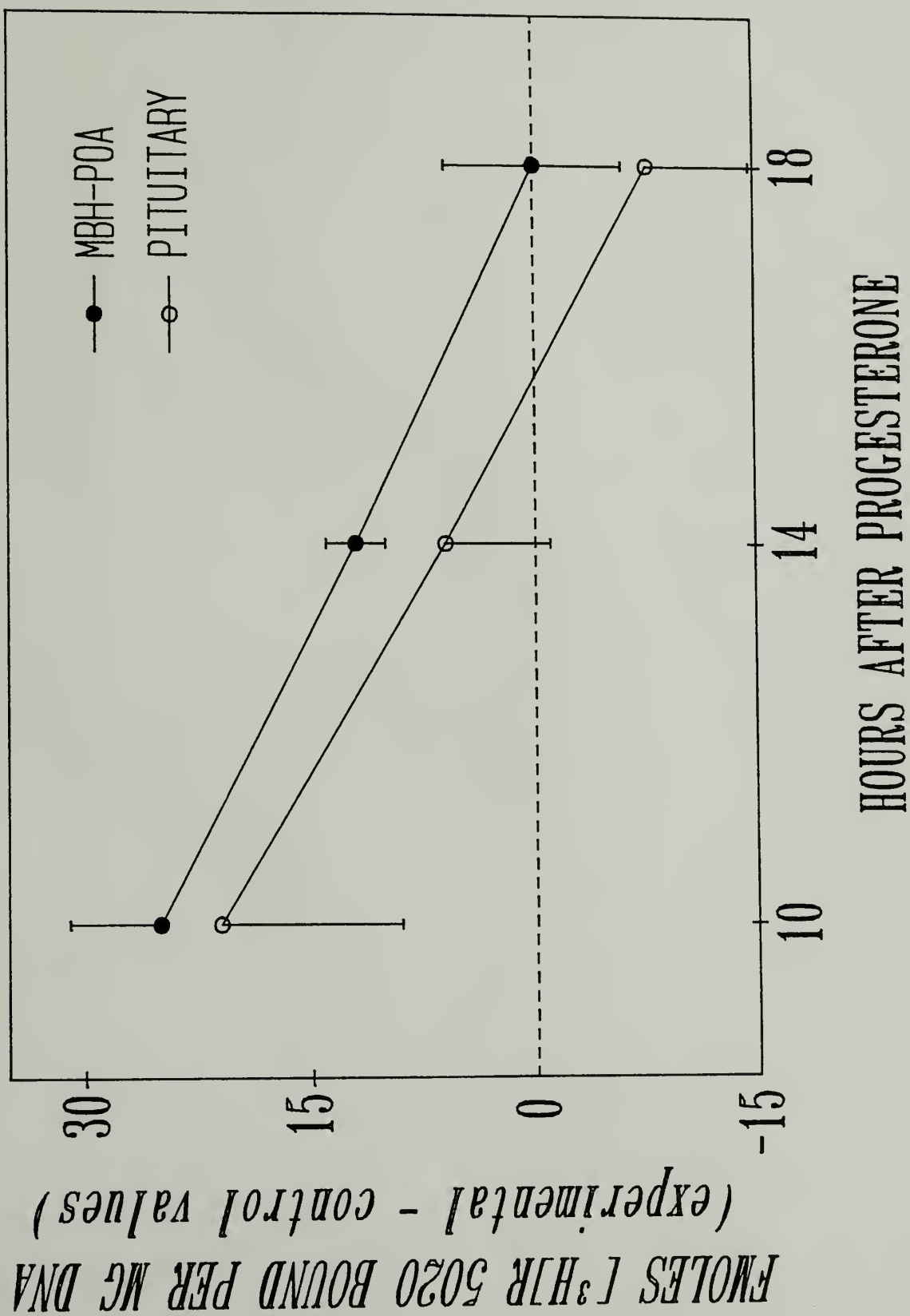
Progesterone-injected animals displayed significantly higher concentrations of nuclear progestin receptors than did oil-injected animals at both 10 h ($t_{10}=3.701$, $p<0.01$; Fig. 6) and 14 h ($t_{10}=4.511$, $p<0.002$) after treatment. By 18 h after treatment, the concentration of nuclear progestin receptors was no longer elevated in the progesterone-injected group ($t_{10}=0.066$, $p>0.8$).

Anterior pituitary

At no time after progesterone-injection was an increase in the concentration of nuclear progestin receptors detectable in the anterior pituitary (10 h: $t_9=1.750$, $p>0.1$; 14 h: $t_{10}=0.820$, $p>0.2$; 18 h: $t_{10}=1.139$, $p>0.2$; Fig. 6).

Figure 6

Nuclear progestin receptor concentrations measured in mediobasal hypothalamus-preoptic area and anterior pituitary. Ovariectomized rats were injected with 500 μ g of progesterone or oil vehicle 44 h after 2 μ g of estradiol benzoate and killed 10, 14, or 18 h later. Values given represent the difference (mean \pm S.E.M.) between receptor levels measured in progesterone-treated and oil-treated animals, n = 5-6 per group.



C H A P T E R V I
EXPERIMENT 4: RU 486 BINDING TO
NUCLEAR PROGESTIN RECEPTORS

Rationale

In experiment 2, RU 486 effectively abbreviated sexual behavior whether injected 2, 6, or 10 h after progesterone treatment. Brown and Blaustein (1986) reported a similar abbreviation of sexual behavior in ovariectomized estrogen-primed guinea pigs when RU 486 was injected 4 h after progesterone treatment. They also reported lower nuclear concentrations of hypothalamic progestin receptors at 2 and 6 h after RU 486-injection. Since experiment 3 revealed the presence of elevated levels of nuclear progestin receptors in rats as late as 14 h after progesterone treatment, perhaps the abbreviated period of sexual behavior seen in experiment 2 was due to RU 486-binding to intracellular progestin receptors, which subsequently lowered the concentration of nuclear receptors in the hypothalamus. As a first step in examining the possible effects of RU 486 on progestin receptors, we attempted to determine if the concentration of nuclear progestin receptors in rats is decreased by the injection of RU 486 as in the case with guinea pigs.

Procedure

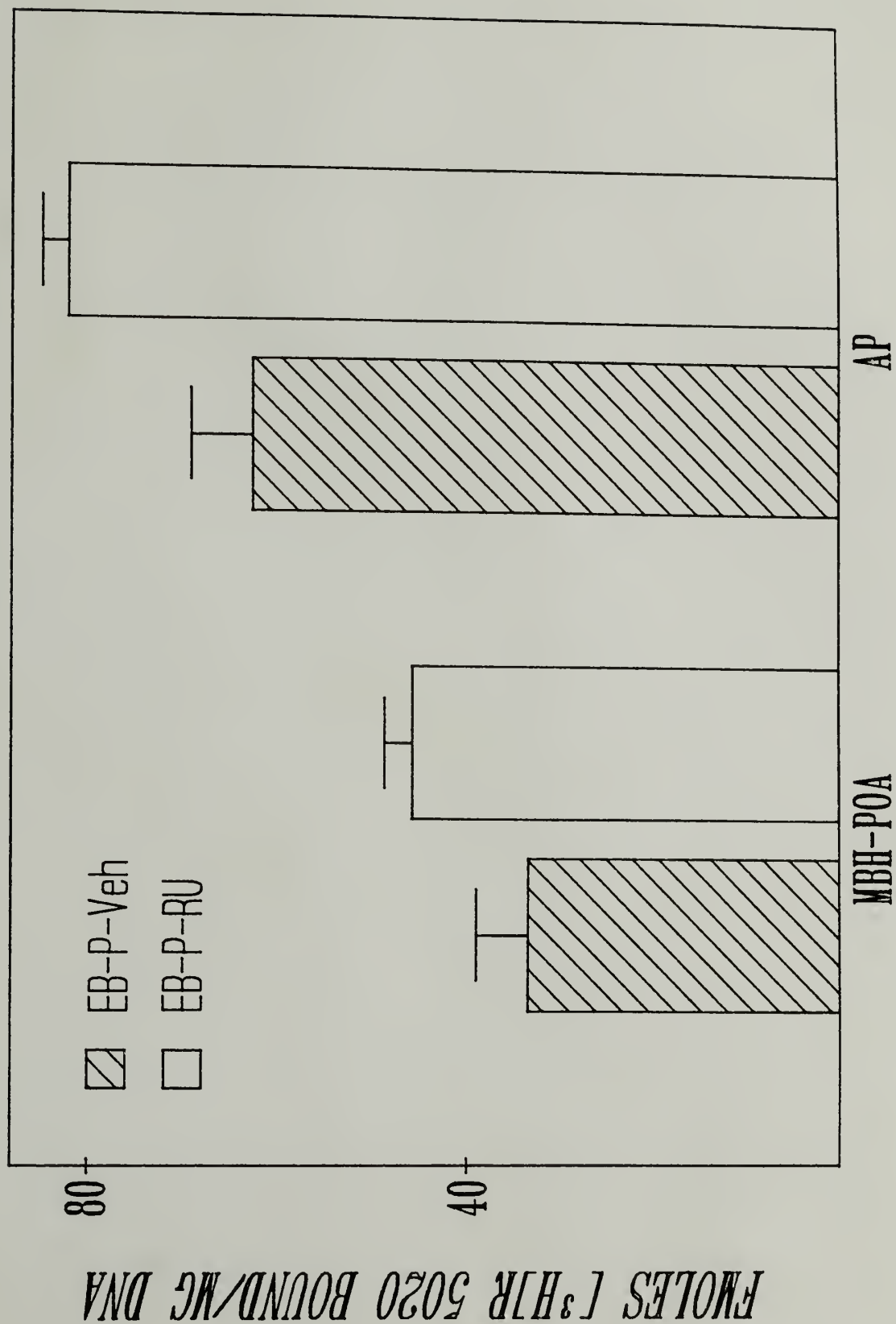
Ovariectomized rats were injected with 500 μ g of progesterone 44 h after receiving 2 μ g of estradiol benzoate. Two hours after progesterone treatment, rats were injected with either 5 mg of RU 486 (EB-P-RU) or oil vehicle (EB-P-Veh). Rats were killed 2 h after injection of either RU 486 or oil and nuclear progestin receptor levels of the mediobasal hypothalamus-preoptic area and anterior pituitary were measured.

Results

Rather than decreasing the concentration of nuclear progestin receptors as has been reported in guinea pigs, RU 486 injection resulted in a trend toward higher levels of nuclear progestin receptors in the mediobasal hypothalamus-preoptic area ($t_7=2.092$, $p<0.1$, Fig. 7) and significantly higher receptor levels in the anterior pituitary ($t_8=2.685$, $p<0.05$).

Figure 7

Effect of RU 486 on the concentration of specifically bound [^3H]R 5020 in nuclear extract of mediobasal hypothalamus-preoptic area and anterior pituitary. Ovariectomized rats were injected with 500 μg of progesterone 44 h after 2 μg of estradiol benzoate. Rats were injected with 5 mg of RU 486 (EB-P-RU) or oil vehicle (EB-P-Veh) 2 h after progesterone treatment and killed 2 h later. Values given represent fmoles [^3H]R 5020 specifically bound per mg DNA, n = 4-6 per group.



C H A P T E R V I I

EXPERIMENT 5: RU 486 EXCHANGE WITH R 5020

Rationale

In experiment 4, the injection of RU 486 tended to increase, rather than decrease, the concentration of specifically bound [^3H]R 5020 in nuclear extracts of the mediobasal hypothalamus-preoptic area and anterior pituitary. This was surprising because similar treatment in guinea pigs causes a decrease in nuclear progestin receptor concentrations in the hypothalamus (Brown and Blaustein, 1986). The purpose of this experiment was to determine if, unlike the case in guinea pigs, [^3H]R 5020 exchanges with RU 486 when incubated at 0°C with RU 486-occupied nuclear progestin receptors from rats.

Procedure

Forty-four hours after the injection of 2 μg of estradiol benzoate, ovariectomized rats were injected with 5 mg of RU 486 (EB-RU) or oil vehicle (EB-Veh). Two hours after injection of either RU 486 or oil, rats were killed and mediobasal hypothalamus-preoptic areas were removed and nuclear progestin receptors were measured.

Results

RU 486-injected animals had higher concentrations of hypothalamic nuclear progestin receptors than oil-injected animals (79 ± 8 vs. 37 ± 8 fmoles [^3H]R 5020 bound/mg DNA; $t_9 = 3.549$, $p < 0.01$; $n = 5-6$ per group), suggesting that [^3H]R 5020 exchanged with RU 486 bound to progestin receptors.

CHAPTER VIII

DISCUSSION

McGinnis et al. (1981) and Ahdieh et al. (1986) reported that ovariectomized estrogen-primed rats injected with progesterone continued to display progesterone-facilitated sexual behavior long after the concentration of hypothalamic nuclear progestin receptors returned to baseline levels. Based on these findings, the hypothesis of this thesis was that the initial increase of progestin receptors observed in the hypothalamic cell nuclear fraction may initiate cellular events that maintain the period of sexual receptivity for hours after nuclear progestin receptor levels return to baseline. However, the results of this thesis offer evidence that the concentration of hypothalamic nuclear progestin receptors in estrogen-primed ovariectomized rats returns to baseline levels approximately 18 h after progesterone injection, or at about the same time that similarly-treated animals cease to display progesterone-induced sexual receptivity. Furthermore, the results of the anisomycin experiment suggest that progesterone-injected rats require ongoing hypothalamic protein synthesis as late as 12 h after progesterone injection in order to continue to exhibit sexual behavior.

In the light of previous reports, the first experiment assumed that the concentration of hypothalamic nuclear progestin receptors returned to baseline levels approximately 10 h after progesterone treatment, and examined whether or not injecting anisomycin at this time would block subsequent sexual behavior. We hypothesized that if sexual behavior was induced by long-lived proteins synthesized in the hypothalamus in response to an elevated concentration of hypothalamic nuclear progestin receptors, then perhaps anisomycin would not block sexual behavior if injected after receptor levels returned to baseline concentrations. However, in experiment 1, progesterone-facilitated sexual behavior was blocked whether anisomycin was injected at 2 or 10 h after progesterone treatment. The same dose of anisomycin also inhibited protein synthesis in the mediobasal hypothalamus by 70% within 2 h of injection, suggesting that sexual receptivity in rats requires ongoing hypothalamic protein synthesis at least as late as 12 h after progesterone injection. It is unlikely that the inhibition by anisomycin was due to incapacitation of the animals, because the same dose of anisomycin which blocked progesterone-induced sexual behavior in experiment 1 failed to block estrogen-induced sexual behavior in a study by Rainbow, Davis, and McEwen (1980). It is more

likely that sexual behavior was blocked in these animals because anisomycin inhibited the synthesis of proteins needed to maintain sexual receptivity. If such were the case, then these behaviorally-relevant proteins probably have a lifespan of less than 2 h, since proteins synthesized prior to the administration of anisomycin did not continue to maintain sexual behavior 2 h later.

In earlier reports, the progesterone antagonist, RU 486, blocked progesterone-facilitated sexual behavior if injected up to 4 h after progesterone in guinea pigs (Brown and Blaustein, 1984a; Brown and Blaustein, 1986) or if administered up to 1 h after progesterone in rats (Etgen and Barfield, 1986). The purpose of the second experiment was to determine if RU 486 would inhibit sexual behavior when injected after the point at which the concentration of hypothalamic nuclear progestin receptors was reported to return to baseline levels. When the progesterone antagonist was injected at either 2 or 6 h after progesterone, sexual behavior was inhibited within 8 h. This came as no surprise, since Brown and Blaustein (1986) reported that RU 486 blocked progesterone-facilitated sexual behavior in guinea pigs if injected when the levels of hypothalamic nuclear progestin receptors were still elevated. Unexpectedly, however, sexual receptivity was also inhibited in experiment 2 when

RU 486 was injected at 10 h after progesterone, which was the time at which the concentration of hypothalamic nuclear progestin receptors was reported to return to baseline levels.

These unexpected results suggested that at 10 h after progesterone treatment, the concentration of hypothalamic nuclear progestin receptors might be elevated enough to maintain sexual behavior, but not be elevated enough for the standard receptor assay to measure. Before cell nuclear progestin receptors were assayed in experiment 3, intact cell nuclei were isolated using a technique which was reported

to increase the yield of nuclear estrogen receptors from rat brain

(Kranzler et al., 1984). Incorporating this technique into the nuclear progestin receptor assay decreased the number of procedural steps, but failed to increase the assay's sensitivity. In spite of this, the third experiment demonstrated that the concentration of hypothalamic nuclear progestin receptors was higher in progesterone-injected animals than in oil-injected controls at both 10 and 14 h after progesterone treatment.

These results contradict a report by McGinnis et al. (1981), and a previous report from our laboratory (Ahdieh et al., 1986), in which the concentration of hypothalamic

nuclear progestin receptors returned to baseline levels approximately 8 h after progesterone treatment. Our laboratory identifies elevated concentrations of nuclear progestin receptors by comparing receptor levels of progesterone-treated animals to the baseline receptor levels of oil-treated animals. In the present study, baseline receptor levels were 20 fmol/mg DNA, whereas in the previous study, baseline levels were 50 fmol/mg DNA. It is possible that the baseline receptor level in the previous study was so high that, when compared to receptor concentrations in progesterone-treated animals, it obscured a small elevation in the level of receptors. On the other hand, McGinnis et al. (1981) reported baseline receptor levels similar to those measured in the present study. However, in the study by McGinnis et al., nuclear progestin receptors were assayed with a low subsaturating concentration of 0.4 nM [^3H]R 5020. In the present experiments, a concentration of 1.5 nM [^3H]R 5020 was used to assay nuclear progestin receptors. A concentration of 0.4 nM [^3H]R 5020 is insufficient to achieve complete saturation of progestin receptors in the nuclear receptor assay (Reading and Blaustein, 1984), so its use may have resulted in an underestimation of receptor content.

As is illustrated in figure 8, the results of experiments 2 and 3 indicate that the concentration of

nuclear progestin receptors in the mediobasal hypothalamus-preoptic area of rats returns to baseline levels at the same time that similarly-treated animals cease to display progesterone-facilitated sexual behavior. These results suggest that rats, like guinea pigs, require elevated levels of hypothalamic nuclear progestin receptors throughout the period of time during which they exhibit progesterone-facilitated female sexual behavior. These results also suggest that at each of the times that RU 486 was injected in experiment 2, the concentration of hypothalamic nuclear progestin receptors was elevated. In light of similar work done in guinea pigs (Brown and Blaustein, 1986), experiment 4 attempted to demonstrate that the abbreviations of sexual behavior observed in experiment 2 corresponded to an RU 486-induced decrease in the concentration of receptors occupied by progesterone in hypothalamic cell nuclei.

RU 486 binds with high affinity to progestin receptors in both rat (Brown and Blaustein unpublished data; Etgen and Barfield, 1986) and guinea pig brain (Brown and Blaustein, 1984a), and causes an accumulation of these receptors in the cell nuclear compartment (Brown and Blaustein, 1986). After demonstrating that the progesterone antagonist did not readily exchange with [^3H]R 5020 at 0-4°C, Brown and Blaustein (1986) went on to

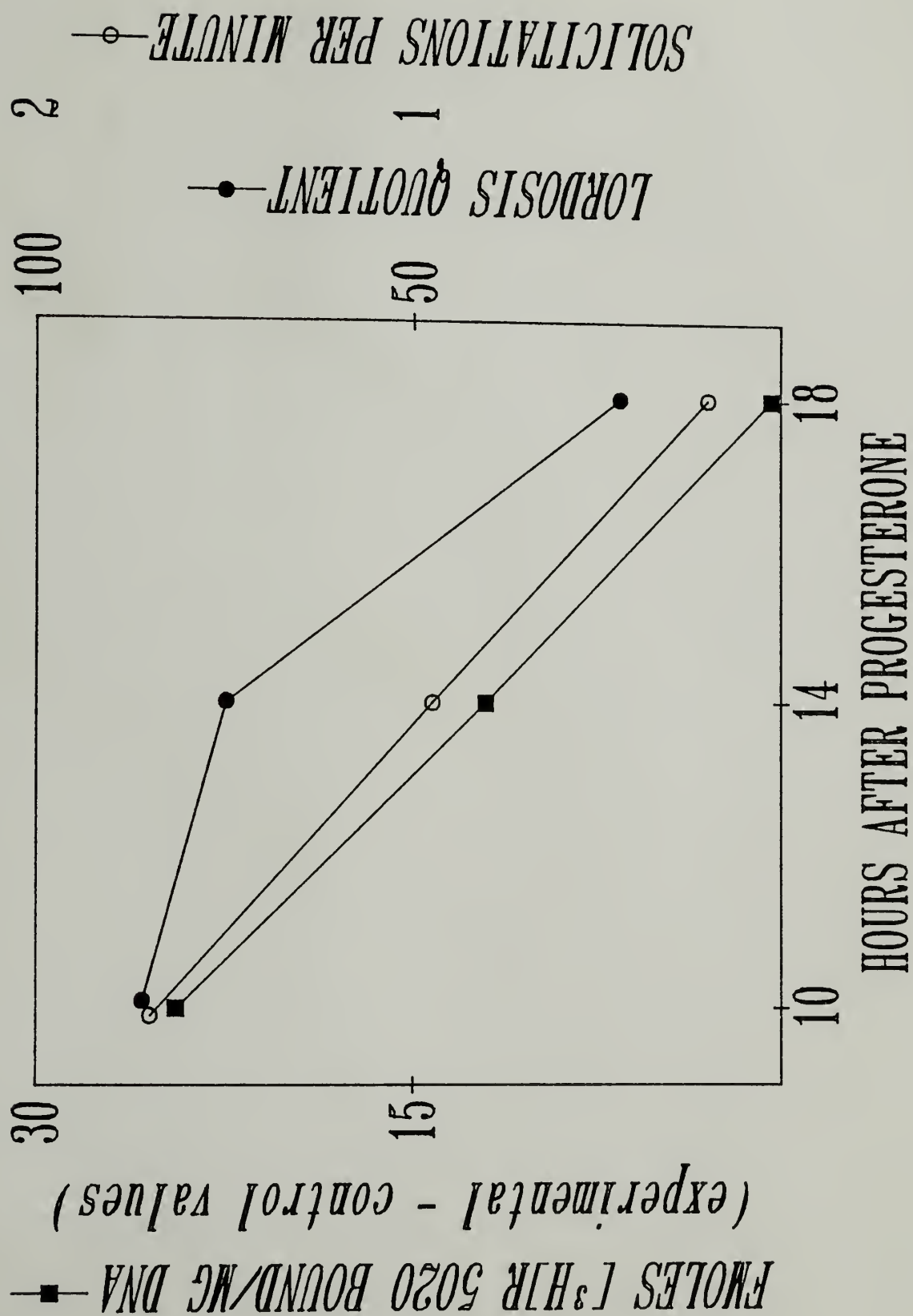
show that the administration of RU 486 after progesterone treatment resulted in a decrease in the level of progesterone-occupied nuclear progestin receptors in guinea pig mediobasal hypothalami. In experiment 4, however, a higher concentration of specifically bound [^3H]R 5020 was measured in the hypothalamic nuclear extracts of RU 486-treated rats than in oil-treated rats when estrogen-primed ovariectomized animals received RU 486 or oil at 2 h after progesterone treatment. Therefore, it appeared that in rats, [^3H]R 5020 exchanged with both progesterone and RU 486 for progestin receptors at 0-4°C, contrary to what was observed in guinea pigs.

The final experiment confirmed that during the course of the overnight incubation at 0-4°C, [^3H]R 5020 did exchange with the RU 486 bound to nuclear progestin receptors from rat hypothalami. Since [^3H]R 5020 bound to receptors whether they were occupied with progesterone or RU 486, we cannot distinguish between progesterone-occupied and RU 486-occupied nuclear progestin receptors in the rat. Therefore, in rats, unlike guinea pigs, the present receptor assay cannot be used to determine if RU 486-induced abbreviation of sexual behavior corresponds to an RU 486-induced decrease in the concentration of receptors occupied by progesterone in hypothalamic cell nuclei.

In summary, the results of this thesis suggest that in rats, as was reported in guinea pigs, female sexual behavior is maintained by the continued action of progesterone in hypothalamic cell nuclei. Rats ceased to display female sexual behavior at the same time that the concentration of progesterone-occupied hypothalamic nuclear progestin receptors returned to baseline levels, a finding which contradicted previous studies. The administration of either a protein synthesis inhibitor or a progestin antagonist as late as 10 h after progesterone injection blocked sexual behavior in these animals. Since the protein synthesis inhibitor blocked sexual behavior 2 h after its injection, this suggests that progesterone activates and maintains sexual behavior by inducing proteins that last for less than 2 h.

Figure 8

Comparison of behavior scores and receptors levels in similarly-treated animals. Solicitations per minute and lordosis quotient scores are from the middle panels of Figures 4 and 5. Hypothalamic nuclear progestin receptor levels are from Figure 6.



BIBLIOGRAPHY

- Ahdieh, H.B., Brown, T.J., Wade, G.N. and Blaustein, J.D., Hypothalamic nuclear progesterin receptors and the duration of sexual receptivity in ovariectomized and ovariectomized-hysterectomized rats, Physiol. Behav., 36 (1985) 211-215.
- Barfield, R.J., Rubin, B.S., Glaser, J.H. and Davis, P.G., Site of action of ovarian hormones in the regulation of oestrous responsiveness in rats. In J. Balthazart, E. Prove and R. Gilles (Eds.), Hormones and Behavior in Higher Vertebrates, Springer-Verlag, Berlin, 1983, pp. 2-17.
- Baulieu, E.E., Contragestion by antiprogestin: a new approach to human fertility control. In R. Porter and M. O'Connor (Eds.), Abortion: Medical Progress and Social Implications, Ciba Foundation Symp. 115, Pittman, London, 1985, pp. 192-210.
- Beach, F.A., Importance of progesterone to induction of sexual receptivity in spayed female rats, Proc. Soc. Exp. Bio. Med., 51 (1942) 369-371.
- Blandau, R.J., Boling, J.L. and Young, W.C., The length of heat in the albino rat as determined by the copulatory response, Anat. Rec., 79 (1941) 453-463.
- Blaustein, J.D., Alteration of sensitivity to progesterone facilitation of lordosis in guinea pigs by modulation of hypothalamic progesterin receptors, Brain Res., 243 (1982a) 287-300.
- Blaustein, J.D., Progesterone in high doses may overcome progesterone's desensitization effect on lordosis by translocation of hypothalamic progesterin receptors, Horms. Behav., 16 (1982b) 175-190.
- Blaustein, J.D. and Feder, H.H., Cytoplasmic progesterin-receptors in guinea pig brain: characteristics and relationship to the induction of sexual behavior, Brain Res., 169 (1979) 481-497.
- Blaustein, J.D. and Feder, H.H., Nuclear progesterin receptors in guinea pig brain measured by an in vitro exchange assay after hormonal treatments that affect lordosis, Endocrinology, 106 (1980) 1061-1069.

- Blaustein, J.D., Finkbohner, R. and Delville, Y., Estrogen-induced and estrogen-facilitated female rat sexual behavior is not mediated by progestin receptors, Neuroendocrinology, 45 (1987) 152-159.
- Blaustein, J.D. and Wade, G.N., Sequential inhibition of sexual behavior by progesterone in female rats: comparison with a synthetic antiestrogen, J. Comp. Physiol. Psych., 91 (1977) 752-760.
- Boling, J.L. and Blandau, R.J., The estrogen-progesterone induction of mating responses in the spayed female rat, Endocrinology, 25 (1939) 359-364.
- Brown, T.J. and Blaustein, J.D., Inhibition of sexual behavior in female guinea pigs by a progestin receptor antagonist, Brain Res., 301 (1984a) 343-349.
- Brown, T.J. and Blaustein, J.D., 1-(o-chlorophenyl)-1(p-chlorophenyl)2,2,2-trichloroethane induces functional progestin receptors in the rat hypothalamus and pituitary gland, Endocrinology, 115 (1984b) 2052-2058.
- Brown, T.J. and Blaustein, J.D., Supplemental progesterone delays heat termination and the loss of progestin receptors from hypothalamic cell nuclei in female guinea pigs, Neuroendocrinology, 39 (1984c) 384-391.
- Brown, T.J. and Blaustein, J.D., Loss of hypothalamic nuclear-bound progestin receptors: factors involved and the relationship to heat termination in female guinea pigs, Brain Res., 358 (1985) 180-190.
- Brown, T.J. and Blaustein, J.D., Abbreviation of the period of sexual behavior in female guinea pigs by the progesterone antagonist RU 486, Brain Res., 373 (1986) 103-113.
- Brown-Grant, K., Exley, D. and Naftolin, F., Peripheral plasma oestradiol and luteinizing hormone concentrations during the oestrous cycle of the rat, J. Endocrinol., 48 (1970) 295-296.
- Burton, K., A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, Biochem. J., 62 (1956) 315-323.

- Collins, V.J., Bolings, J.L., Dempsey, E.W. and Young, W.C., Quantitative studies of experimentally induced sexual receptivity in spayed guinea pigs, Endocrinology, 23 (1938) 188-196.
- Dempsey, E.W., Hertz, R. and Young, W.C., The experimental induction of oestrus (sexual receptivity) in the normal and ovariectomized guinea pig, Amer. J. Physiol., 116 (1936) 201-209.
- Edwards, D.A. and Pfeifle, J.K., Hormonal control of receptivity, proceptivity and sexual motivation, Physiol. Behav., 30 (1983) 437-443.
- Etgen, A.M. and Barfield, R.J., Antagonism of female sexual behavior with intracerebral implants of antiprogesterin RU 38486: correlation with binding to neural progesterin receptors, Endocrinology, 119 (1986) 1610-1617.
- Feder, H.H., Goy, R.W. and Resko, J.A., Progesterone concentrations in the peripheral plasma of cyclic rats, J. Physiol., 191 (1967) 136P-137P.
- Feder, H.H., Landau, I.T., Marrone, B.L. and Walker, W.A., Interactions between estrogen and progesterone in neural tissues that mediate sexual behavior of guinea pigs, Psychoneuroendocrinology, 2 (1977) 337-347.
- Feder, H.H. and Marrone, B.L., Progesterone: its role in the central nervous system as a facilitator and inhibitor of sexual behavior and gonadotropin release, Ann. N.Y. Acad. Sci., 286 (1977) 331-354.
- Feder, H.H., Resko, J.A. and Goy, R.W., Progesterone concentrations in the arterial plasma of guinea-pigs during the oestrus cycle, J. Endocrinol., 40 (1968a) 505-513.
- Feder, H.H., Resko, J.A. and Goy, R.W., Progesterone levels in the arterial plasma of pre-ovulatory and ovariectomized rats, J. Endocrinol., 41 (1968b) 563-569.
- Gagne, D., Pons, M. and Philibert, D., RU 38486: a potent antiglucocorticoid in vitro and in vivo, J. Steroid Biochem., 23 (1985) 247-251.

- Gasc, J.M., Renoir, J.M., Radanyi, C., Joab, I., Tuohimaa, P. and Baulieu, E.E., Progesterone receptor in the chick oviduct: an immunohistochemical study with antibodies to distinct receptor components, J. Cell Biol., 99 (1984) 1193-1201.
- Glaser, J.H. and Barfield, R.J., Blockade of progesterone-activated estrous behavior in rats by intracerebral anisomycin is site specific, Neuroendocrinology, 38 (1984) 337-343.
- Glaser, J.H., Rubin, B.S. and Barfield, R.J., Onset of the receptive and proceptive components of feminine sexual behavior in rats following the intravenous administration of progesterone, Horms. Behav., 17 (1983) 18-27.
- Gravanis, A., Schaison, G., George, M., de Brux, J., Satyaswaroop, P.G., Baulieu, E.E. and Robel, P., Endometrial and pituitary responses to the steroidal antiprogesterin RU 486 in postmenopausal women, J. Clin. Endocrinol. Metab., 60 (1985) 156-163.
- Hansen, S. and Sodersten, P., Reversal of progesterone inhibition of sexual behavior in ovariectomized rats by high doses of progesterone, J. Endocrinol., 80 (1979) 381-388.
- Hardy, D.F. and DeBold, J.F., Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat, Physiol. Behav., 7 (1971) 643-645.
- Joslyn, W.D. and Feder, H.H., Facilitatory and inhibitory effects of supplementary estradiol benzoate given to ovariectomized, estrogen-primed guinea pigs, Horms. Behav., 2 (1971) 307-314.
- Kato, J. and Onouchi, T., Specific progesterone receptors in the hypothalamus and anterior hypophysis of the rat, Endocrinology, 101 (1977) 920-928.
- King, W.J. and Greene, G.L., Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells, Nature (Lond.), 307 (1984) 745-747.

- Kranzler, J.D., Jones, E.E., MacLusky, N.J., Sakamoto, H., Naftolin, F., An exchange assay for the measurement of cell nuclear estrogen receptors in microdissected brain regions, J. Neurochem., 43 (1984) 895-898.
- Kubli-Garfias, C. and Whalen, R.E., Induction of lordosis behavior in female rats by intravenous administration of progestins, Horm. Behav., 9 (1977) 380-386.
- Lisk, R.D., A comparison of the effectiveness of intravenous, as opposed to subcutaneous, injection of progesterone for the induction of estrous behavior in the rat, Can. J. Biochem. Physiol., 38 (1960) 1381-1383.
- Luttge, W.G., and Hughes, J.R., Intracerebral implantation of progesterone: re-examination of the brain sites responsible for facilitation of sexual receptivity in estrogen-primed ovariectomized rats, Physiol. Behav., 17 (1976) 771-775.
- MacLusky, N.J. and McEwen, B.S., Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others, Nature (Lond.), 274 (1978) 276-278.
- Madlafousek, J. and Hlinak, Z., Sexual behavior of the female laboratory rat: inventory, patterning, and measurement, Behavior, 63 (1977) 129-174.
- Marrone, B.L., Rodriguez-Sierra, J.F. and Feder, H.H., Lordosis: inhibiting effects of progesterone in the female rat, Horms. Behav., 8 (1977) 391-402.
- McGinnis, M.Y., Parsons, B., Rainbow, T.C., Krey, L.C. and McEwen, B.S., Temporal relationship between cell nuclear progestin receptor levels and sexual receptivity following intravenous progesterone administration, Brain Res., 218 (1981) 365-371.
- Meyerson, B., Latency between intravenous injection of progestins and the appearance of estrous behavior in estrogen-treated ovariectomized rats, Horm. Behav., 3 (1972) 1-9.
- Moguilewsky, M. and Philibert, D.J., RU 38486: potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation, J. Steroid Biochem., 20 (1984) 271-276.

- Moguilewsky, M. and Raynaud, J.P., Estrogen-sensitive progestin-binding sites in the female rat brain and pituitary, Brain Res., 164, (1979a) 165-175.
- Moguilewsky, M. and Raynaud, J.P., The relevance of hypothalamic and hyphophyseal progestin receptor regulation in the induction and inhibition of sexual behavior in the female rat, Endocrinology, 105 (1979b) 516-522.
- Morin, L.P., Progesterone: inhibition of rodent behavior, Physiol. Behav., 18 (1977) 701-715.
- Morin, L.P. and Feder, H.H., Hypothalamic progesterone implants and facilitation of lordosis behavior in estrogen-primed ovariectomized guinea pigs, Brain Res., 70 (1974) 81-93.
- Nadler, R.D., A biphasic influence of progesterone on sexual receptivity of spayed female rats, Physiol. Behav., 5 (1970) 95-97.
- Nequin, L.G., Alvarez, J. and Schwartz, N.B., Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4 day and 5 day estrous cycles in the rat, Biol. Reprod., 20 (1979) 659-670.
- Parsons, B., MacLusky, N.J., Krey, L., Pfaff, D.W. and McEwen, B.S., The temporal relationship between estrogen-inducible progestin receptors in the female rat brain and the time course of estrogen activation of mating behavior, Endocrinology, 107 (1980) 774-779.
- Parsons, B., Rainbow, T.C., MacLusky, N.J. and McEwen, B.S., Progestin receptor levels in rat hypothalamic and limbic nuclei, J. Neurosci., 2 (1982a) 1446-1452.
- Parsons, B., Rainbow, T.C., Pfaff, D.W. and McEwen, B.S., Hypothalamic protein synthesis essential for the activation of the lordosis reflex in the female rat, Endocrinology, 110 (1982b) 620-624.
- Philibert, D., Deraedt, R., Teutsch, G., Tournemine, C. and Sakiz, E., RU 38486--a new lead for steroidal antihormones. In Abstr. 64th Annu. Meet. of the Endocrine Soc., The Endocrine Society, San Fransisco, 1982, p. 246.

- Powers, J.B., Facilitation of lordosis in ovariectomized rats by intracerebral progesterone implants, Brain Res., 48 (1972) 311-325.
- Quadagno, D.M., McCullough, J. and Langan, R., The effect of varying amounts of exogenous estradiol benzoate on estrous behavior in the rat, Horm. Behav., 3 (1972) 175-179.
- Rabe, T., Kiesel, L. and Runnebaum, B., Antiprogestins. In D. Ganten and D. Pfaff (Eds.), Current Topics in Neuroendocrinology, Vol. 5, Springer-Verlag, Berlin, 1985, pp. 1-29.
- Rainbow, T.C., Davis, P.G. and McEwen, B.S., Anisomycin inhibits the activation of sexual behavior by estradiol and progesterone, Brain Res., 194 (1980) 548-555.
- Rainbow, T.C., McGinnis, M.Y., Davis, P.G. and McEwen, B.S., Application of anisomycin to the lateral ventromedial nucleus of the hypothalamus inhibits the activation of sexual behavior by estradiol and progesterone, Brain Res., 233 (1982a) 417-423.
- Rainbow, T.C., McGinnis, M.Y., Krey, L.C. and McEwen, B.S., Nuclear progestin receptors in rat brain and pituitary, Neuroendocrinology, 34 (1982b) 426-432.
- Rauch, M., Loosfelt, H., Philibert, D. and Miligrom, E., Mechanism of action of an antiprogesterone, RU486, in the rabbit endometrium: effects of RU486 on the progesterone receptor and on the expression of the uteroglobin gene, Eur. J. Biochem., 148 (1985) 213-218.
- Reading, D.S. and Blaustein, J.D., The relationship between heat abbreviation and neural progestin receptors in female rats, Physiol. Behav., 32 (1984) 973-981.
- Ross, J., Claybaugh, C., Clemens, L.G. and Gorski, R.A., Short latency induction of estrous behavior with intracerebral gonadal hormones in ovariectomized rats, Endocrinology, 89 (1971) 32-38.
- Rubin, B.S. and Barfield, R.J., Progesterone in the ventromedial hypothalamus facilitates estrous behavior in ovariectomized, estrogen-primed rats, Endocrinology, 113 (1983) 797-804.

- Sheridan, P.J., Buchanan, J.M., Anselmo, V.C. and Martin, P.M., Equilibrium: the intracellular distribution of steroid receptors, Nature (Lond.), 282 (1979) 579-582.
- Shivers, B.D., Harlan, R.E., Parker, C.R. and Moss, R.L., Sequential inhibitory effect of progesterone on lordotic responsiveness in rats: time course, estrogenic nullification, and actinomycin-D insensitivity, Biol. Reprod., 23 (1980) 963-973.
- Sodersten, P. and Eneroth, P., Serum levels of oestradiol-17 β and progesterone in relation to sexual receptivity in intact and ovariectomized rats, J. Endocrinol., 89 (1981) 45-54.
- Spilman, C.H., Gibson, R.E., Beuving, D.C. and Campbell, J.A., Progesterin and antiprogesterin effects on progesterone receptor transformation, J. Steroid Biochem., 24 (1986) 383-389.
- Thornton J.E., Nock, B., McEwen, B.S. and Feder, H.H., Estrogen induction of progestin receptors in microdissected hypothalamic and limbic nuclei of female guinea pigs, Neuroendocrinology, 43 (1986) 182-188.
- Wallen, K. and Thornton, J.E., Progesterone and duration of heat in estrogen-treated ovariectomized guinea pigs, Physiol. Behav., 22 (1979) 95-97.
- Welshons, W.V., Krummel, B.M. and Gorski, J., Nuclear localization of unoccupied receptors for glucocorticoids, estrogens, and progesterone in GH₃ cells, Endocrinology, 117 (1985) 2140-2147.
- Welshons, W.V., Liebermann, M.E. and Gorski, J., Nuclear localization of unoccupied oestrogen receptors, Nature (Lond.), 307 (1984) 747-749.
- Yanase, M. and Gorski, R.A., Sites of estrogen and progesterone facilitation of lordosis behavior in the spayed rat, Biol. Reprod., 15 (1976) 536-543.
- Young, W.C., Dempsey, C., Hagquist, C.W., and Boling, J.L., Sexual behavior and sexual receptivity in the female guinea pig, J. Comp. Psychol., 27 (1939) 49-68.
- Zucker, I., Facilitory and inhibitory effects of progesterone on sexual responses of spayed guinea pigs, J. Comp. Physiol. Psychol., 62 (1966) 376-381.

