NEUROTRANSMITTERS INVOLVED IN THE SUPPRESSIVE EFFECT OF SUCKLING ON PLASMA LUTEINIZING HORMONE

by

Wade Clay Richardson

Animal Science

Submitted in Partial Fulfillment of the Requirements of the

University Undergraduate Fellows Program

1976 - 1977

Approved by:

Dr. P. G. Harms

May 1977

ABSTRACT

Neurotransmitters Involved in the Suppressive Effect of Suckling on Plasma Luteinizing Hormone

The involvement of neurotransmitters in the inhibitory effect of suckling on plasma LH was investigated. The effect of suckling on the plasma LH levels was studied when the activity of the neurotransmitters, dopamine or serotonin was blocked. Ovariectomized rats, suckling six pups were used for the experiments. Three blood samples were collected from each rat. The first sample was obtained after continuous nursing of the mothers. The pups were then removed for 12 hours and a second sample was taken. The pups were put back with their respective mothers for 12 hours and the third sample was taken. Drugs were administered so that the specific effect of each neurotransmitter was blocked during the last 12 hour period of suckling. There was no significant effect on the suppressive effect of suckling on LH levels when dopamine activity was blocked. With the blockage of serotonin activity plasma LH levels failed to increase with pup removal. These results suggest that the neurotransmitter dopamine may not be involved but that serotonin may be involved in the effect of suckling on LH.

ACKNOWLEDGEMENTS

The author extends his most sincere gratitude to Dr. P. G. Harms for his many hours of guidance throughout this research project.

Appreciation is also extended to Bob Owens for his assistance with experimental procedures and to Mrs. Martha Wright for her analysis of blood samples.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Postpartum anestrus, failure to return to estrus following parturition, is a major problem associated with the production of beef cattle. This phenomena impairs the reproductive efficiency of cattle and hence increases the cost of production. The problem is affected by nutritional level and age, but appears to result primarily from the suckling stimulus of the young. Any steps toward overcoming the detrimental effect of suckling would be of great economic importance to all cattlemen.

The mechanism by which the suckling stimulus travels through the nervous system to bring about the effect is not fully understood. It is believed that the stimulus moves to the brain, more specifically the hypothalamus, and impairs the release from the anterior pituitary LH, necessary for ovulation. At ovulation an ovum is released from a mature follicle on the ovary and becomes available for fertilization. Low levels of LH prevent ovulation and there by may impair reproduction.

The objective of this research was to evaluate the suppressive effect of suckling on plasma LH when the action of the neurotransmitters dopamine or serotonin was blocked by drugs. Rats were used as the experimental animal since this species exhibits the anestrus phenomenum and is well suited for such studies.

The citations on the following pages follow the style of the *Journal of Animal Science*.

LITERATURE REVIEW

Hypothalamic Regulation of Pituitary LH Release

As reviewed by Convey (1973), the hypothalamus controls the release of pituitary hormones via neurohormones called releasing hormones. Luteinizing Hormone Releasing Hormone (LHRH), in response to the stimulus of certain neurotransmitters, travels down the portal vessels from the hypothalamus to the anterior pituitary gland. Consequently LH is released, causing a dramatic increase of LH in the blood resulting in ovulation.

Effect of Suckling on Reproduction

Minaguchi and Meites (1966) reported that suckling acts on the hypothalamus to depress release of LHRH resulting in the inhibition of pituitary LH release. These data in combination with that of Mena *et al.* (1975) on hypothalmic neurotransmitter levels in the rat suggest a correlation between the plasma levels of LH and hypothalmic levels of the neurotransmitters, dopamine and serotonin. Mena showed a depletion in hypothalmic levels of serotonin and dopamine within five minutes following the onset of suckling. The depletion lasted as long as the stimulus was maintained. The above results suggest that the reduction of LH levels may be mediated through a system involving the neurotransmitters serotonin or dopamine.

Effect of Suckling on LH Levels

Suckling has an effect of the release of LH from the pituitary. It was first reproted by McCann *et al.* (1961) that plasma LH levels are decreased in ovariectomized rats during lactation. Similarly, Hammons

et al. (1973) found that ovariectomized rats with nine pups had lower levels of LH during the first days of postpartum than did rats nursing two pups.

With the suckling stimulus removed for 12 hours it was found by Harms *et al.* (1977) that there was a highly significant increase in the plasma LH levels. With replacement of the suckling stimulus for 12 hours plasma LH decreased dramatically.

MATERIALS AND METHODS

Experimental Animals

Sprague-Dawley strain female rats (Simonsen Labs, Gilroy, Calif.) were housed individually in a rat battery. The environment was maintained at approximately 25° C. with automatically controlled lighting at 14 hours light and 10 hours dark. The diet consisted of Purina Lab Chow and tap water *ad libitum*.

Experimental Regimen

Day	Time	Treatment
0		Parturition
2		Ovariectomy
7	0700	Pups removed, blood sample, injection if administered
	1900	Pups returned, blood sample, injection if administered
8	0700	Blood sample

Table 1. Treatment of the Animals

Parturition was considered as day 0. On day 2 a bilateral ovariectomy was performed to remove any ovarian interference of the hormone levels. The number of pups was evened to six on each mother. On day seven of the experimental procedure at 0700 a blood sample was taken. In the investigation of serotonin, a subcutaneous injection of 25 mg/100 gm of Parachlorophenylalanine which inhibits the synthesis of serotonin was administered and the pups were removed. The pups were maintained away from the females for 12 hours so as to eliminate the suckling stimulus. At 1900 a second blood sample was taken and the pups were returned to their respective mothers. Also at this time in the investigation of dopamine, a subcutaneous injection of pimozide, which blocks dopamine receptor sites, was given at the dosage of 0.63 mg/kg. Twelve

hours later the final blood sample was taken. The control group consisted of ovariectomized animals that were not treated with drugs, yet underwent the same pup removal regimen.

Blood Collection and Analysis

Blood samples were taken from the juglar veins of lightly anesthesized rats. The juglar vein of each rat was exposed by making a small incision through the skin of the neck. The juglar vein was exposed and approximately one milliter of blood was withdrawn. The syringe was heparinized to prevent clotting of the blood.

The blood samples were then centrifuged and the plasma was removed. The samples were then stored at -20° C. until hormone analysis. The plasma level of LH was determined by the validated radio immunoassay, described by Niswender *et al.* (1968). The data were analyzed by the paired-T statistical analysis.

RESULTS

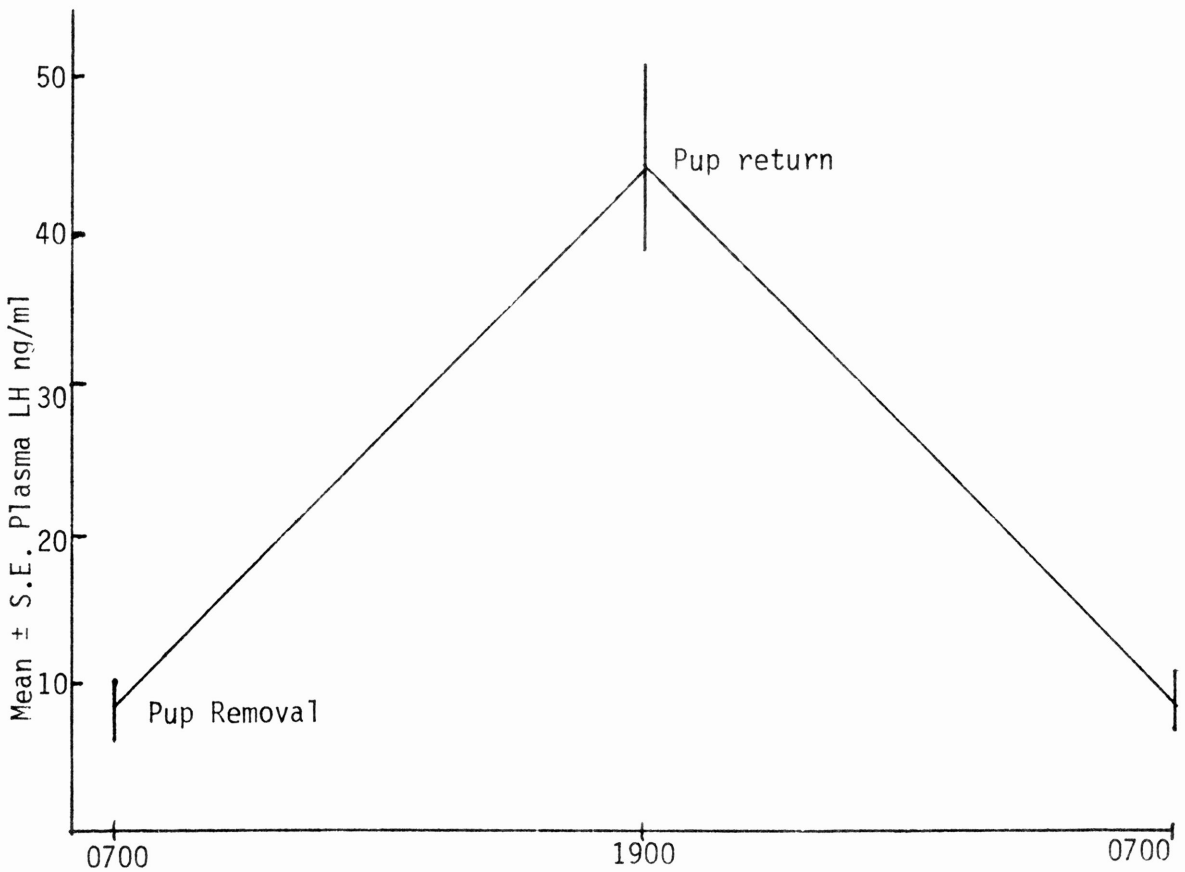

Figure 1. Effect of Suckling on LH

Figure 1 presents the data obtained from the control group of animals. Within 12 hours the level of LH in the plasma increased significantly from a mean level of 8.0 ng/ml to 44.4 ng/ml due to removal of the suckling stimulus. When the pups were replaced the level decreased to 8.7 ng/ml.

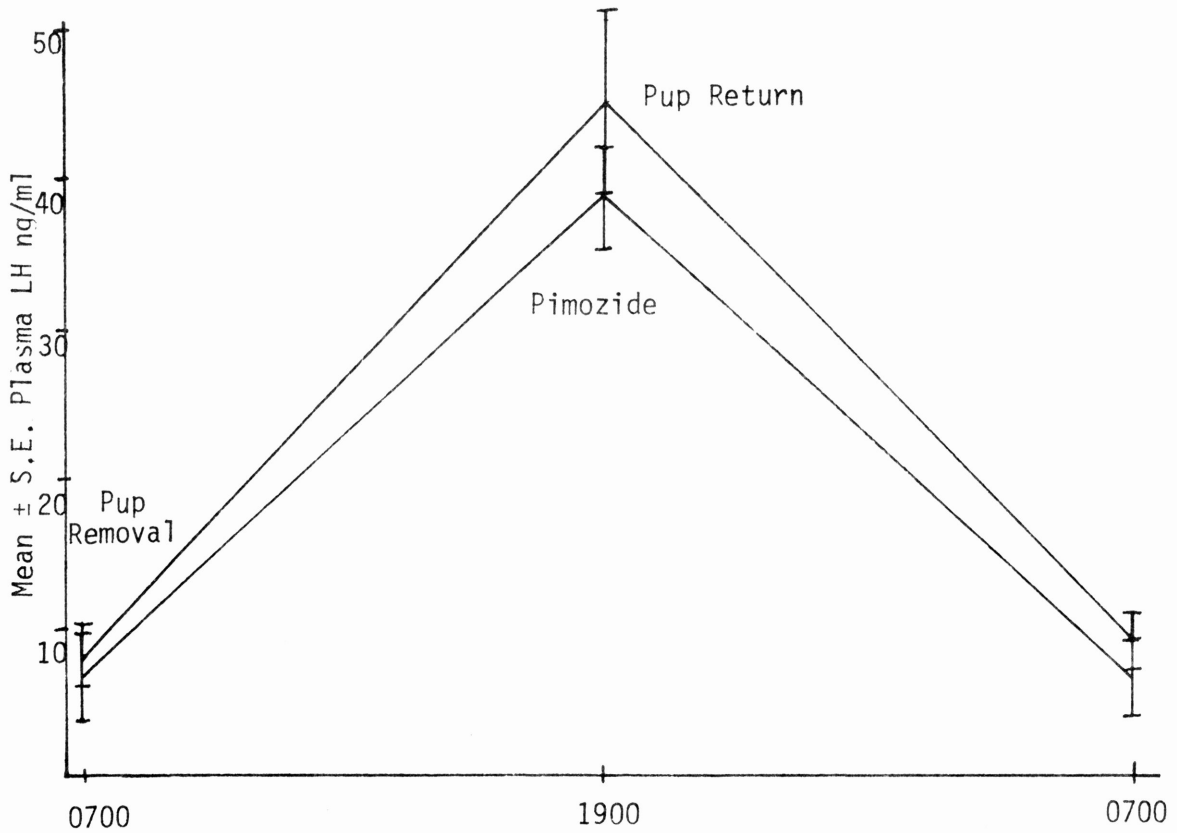

Figure 2. Effect of Pimozide on LH

Figure 2 illustrates the results of the pimozide treatment to check for dopamine envolvement in the mediation of the suckling stimulus through the hypothalamus. The mean level of plasma LH on day 7 at 0700 was 6.5 ng/ml. This rose to a level of 38.5 ng/ml 12 hours after the pups were removed, coinciding with the control group. At this point the six animals were treated with pimozide, and the pups were returned. Within 12 hours the mean level of LH had returned to the initial level of 6.3 ng/ml. These data suggest a lack of dopamine involvement in the transmission of the stimulus from suckling.

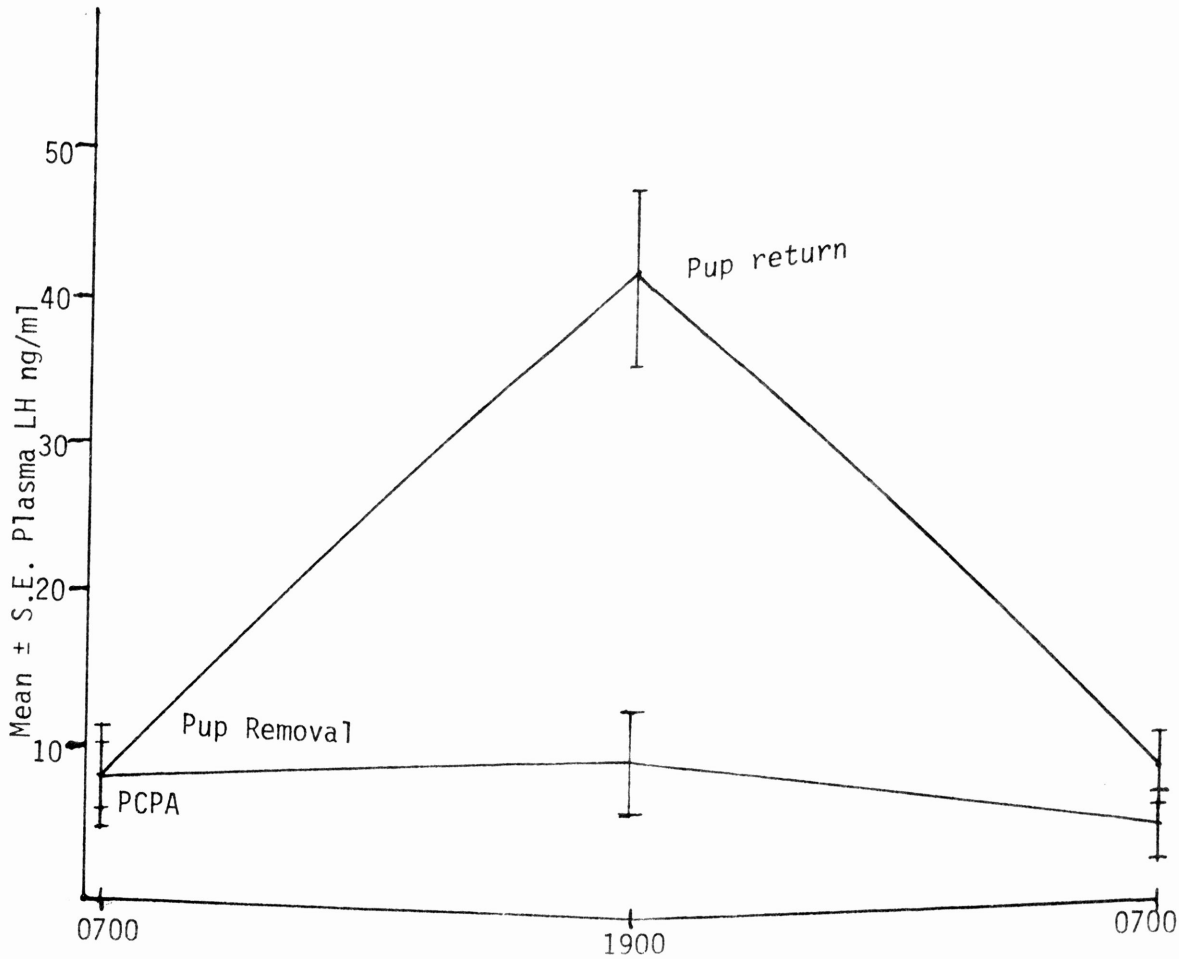

Figure 3. Effect of PCPA on Suckling LH Levels

Figure 3 presents the data that were obtained to determine possible serotonin mediation of the suckling stimulus. As in the previous two trials, the original level of plasma LH was low (8.5 ng/ml) in suckled, lactating, ovariectomized rats. At 0700 on day 7 the animals were treated with PCPA to block serotonin synethesis, and the pups were removed. Twelve hours later the mean level of LH increased only 2 mg to 10.8 ng/ml. The latter use was significantly lower than in the control group in which LH rose to 44 ng/ml after the pups were returned to the mother for 12 hours the plasma LH was 4.6 ng/ml.

DISCUSSION OF RESULTS

The suppressive effect of suckling on plasma LH observed in this study is in agreement with previous reports. (Hammons *et al.*, Harms *et al.*, and McCann *et al.*). In addition the results suggest possible neurotransmitter involvement in the suckling effect.

When transmission was impaired through blockage of dopamine receptor sites with pimozide suckling lowered plasma LH as in the controls. If the decrease in dopamine levels in the hypothalamus at suckling was related to the reduction in plasma LH, then there should have been a differentiation in the control and pimozide treated groups in this study. Consequently the data suggest that dopamine is not involved in the mechanism of reduced pituitary release of LH during the postpartum.

In addition to the apparent lack of dopamine involvement, our results provide evidence for a possible role of serotonin in the suppressive effect of suckling on plasma LH. When serotonin synthesis was blocked by PCPA the increase in LH which normally occurs with the removal of the suckling stimulus failed to occur. Such an effect suggests that serotonin synthesis is necessary for the LH increase in the absence of suckling. It is possible to speculate then that during suckling, serotonin synthesis, which may be required for LH release, may be impaired. Further studies are required before it is possible to make a definitive conclusion concerning serotonin involvement in the effect of suckling in reproductive function.

LITERATURE CITED

Convey, E. M., Neuroendocrine Relationships in FArm Animals: A Rview. Journal of Animal Science. 37:745, 1973.

Hammons, J., M. Velasco, and I. Rothchild. 1973. Effect of sudden withdrawal or increase of suckling on serum LH levels in ovariectomized postpartient rats. Endocrinol. 92:206.

Harms, P. G., Fleeger, and B. Owens. 1977. Federation Proceedings: 36. 106. (Abstr.)

McCann, S. M., T. Graves and S. Taleisnik. 1961. The effect of lactation on plasma LH. Endocrinol. 68:873. (Abstr.)

Mena, F., A. Enjalbert, L. Carbonell, M. Priam, and C. Kordon. 1975. Effect of Suckling on Plasma Prolactin and Hypothalmic Monoamine Levels in the Rat. Endocrinology. 99:445, 1976.

Minaguchi, H. and J. Meites, Endocrinology 80:603. 1967.

Niswender, G. D., L. Riechert, Jr., A. R. Midgley and A. V. Nalbanon. 1969. Radioimmunoassay for bovine and ovine luteinizing hormones. Endocrinol. 84:1166.

APPENDIX TABLE 1. EFFECT OF PUP REMOVAL ON LH

Time	Day 7, 0700	Day 7, 1900	Day 8, 0700
	1.7	66.0	1.7
	6.6	23.1	13.2
	16.5	52.8	16.5
	9.9	39.6	13.2
	2.6	33.0	3.3
	16.5	69.3	6.6
	5.1	24.4	11.4
	5.3	46.8	3.3
Mean	8.0	44.4	8.7
Standard Deviation	5.8	17.6	5.6
Standard Error	2.0	6.2	2.0

APPENDIX TABLE 2. EFFECT OF PIMOZIDE ON LH

Time	Day 7, 0700	Day 7, 1900	Day 8, 0700
	10.4	28.9	13.4
	0.5	36.6	0.5
	0.5	29.1	0.9
	4.1	48.4	6.5
	15.4	41.5	13.7
	8.1	46.6	2.5
Mean	6.5	38.5	6.3
Standard Deviation	5.9	8.4	6.0
Standard Error	2.4	3.4	2.4

APPENDIX TABLE 3. EFFECT OF PCPA ON LH

Time	Day 7, 0700	Day 7, 1900	Day 8, 0700
	3.3	19.8	2.6
	3.3	3.3	2.0
	16.5	13.2	2.0
	16.5	1.0	13.2
	3.0	16.5	3.3
Mean	8.5	10.8	4.6
Standard Deviation	7.3	8.2	4.8
Standard Error	3.3	3.7	2.1