ADRENALECTOMY MODIFIES THE PROLACTIN RESPONSE TO MORPHINE AND NALOXONE

by

Janice Marie Fiori

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ABSTRACT

ADRENALECTOMY MODIFIES THE PROLACTIN RESPONSE TO MORPHINE AND NALOXONE

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Janice M. Fiori

The interactions of the adrenal glucocorticoids and the opiates in the control of prolactin (PRL) secretion were studied in adrenalectomized male rats. Animals were implanted with an indwelling atrial cannula for blood sampling and drug injection. Injection of morphine (3mg/kg,IV) induced a significantly higher rise of plasma PRL in adrenalectomized than in sham-operated rats. Naloxone (1mg/kg,IV) did not suppress basal PRL values in adrenalectomized rats. However, NAL blocked the MOR induced rise in plasma prolactin in adrenalectomized animals. Corticosterone replacement by subcutaneously implanted pellets significantly reduced the morphine-induced rise of prolactin observed in adrenalectomized rats. These results suggest that the adrenal cortex influences the actions of morphine and naloxone on prolactin secretion. The mechanisms by which the adrenal cortical hormones interact with the opiates remain to be investigated.

Dedication

This volume is dedicated to my husband, Jim, for his patience and encouragement. I am also very grateful to my parents, Eugene and Catherine Trapp, and my brothers, Patrick, Timothy, Michael, and Christopher for their continual interest and support.

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I wish to thank my fellow laboratory associates, Paul E. Gottschall, Dr. Paul W. Sylvester, Dr. Lloyd J. Forman, Dr. Vincent Hylka, and Dr. Karen Briski, for help in performing the surgeries and the experiments. I am grateful to Vince and his wife Sharon for their "open-apartment" policy and hospitality.

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Dr. Joseph Meites, in serving as my major professor instilled in me his own high standards, not only in scientific endeavors, but also in interactions with colleagues and in personal development.

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Introduction

The present study investigated the possible interactions of the opiates and the glucocorticoids in the control of prolactin (PRL) secretion. The opiates and glucocorticoids have opposing effects on PRL release. Opiates increase PRL secretion; glucocorticoids inhibit PRL secretion. If the opiates and glucocorticoids interact to maintain PRL control, then removal of one of the components (glucocorticoids) would be expected to produce an abberrance in the normal response to the second component (opiates). Therefore, the response of PRL to morphine (MOR) and naloxone (NAL) administration in adrenalectomized rats was investigated. This experiment was repeated at different intervals following adrenalectomy to determine if the changes in β-endorphin (β-END) and adrenocorticotropin (ACTH) which occur after ADX would influence the PRL response to MOR and NAL.

I. LITERATURE REVIEW

A. Hypothalamic Control of Prolactin Secretion

There is much evidence which suggests that the hypothalamus exerts an inhibitory influence on the release of PRL from the pituitary. For instance, removal of hypothalamic influence on the pituitary by severing the portal vessels, or by transplantation of the pituitary to an extra-sellular site results in increased PRL release (Everett, 1954). Also, hypothalamic extracts can inhibit the release of PRL *in vitro* (Talwalker *et al.*, 1963) and *in vivo* (Grosvenor *et al.* 1965). The hypothalamic inhibition of PRL release from the pitiutary is due primarily to dopamine, which is released from tuberoinfundibular neurons into the portal blood system and acts at dopamine receptors on the lactotrophs of the pituitary (Koch *et al.*, 1970). Drugs that block dopamine action, such as reserpine, a catecholamine depletor, and haloperidol, a dopamine receptor blocker, increase plasma PRL. Dopamine receptor agonists, such as apomorphine, bromocryptine, and piribidel, lower plasma PRL levels (Lu *et al.*, 1971; Mueller *et al.*, 1976).

Meites *et al.* (1960) reported that hypothalamic extracts could stimulate PRL release. After the synthesis of thyrotropin releasing hormone (TRH), it was discovered that TRH released PRL in addition to TSH (Tashijan *et al.*,1971). However, TRH cannot account for all release of PRL by hypothalamic extracts, suggesting there is as yet another undiscovered PRL releasing factor (PRF).

Serotonin (5-HT) has also been reported to stimulate release of PRL. Intraventricular injection of serotonin (Kamberi et al., 1971), or intravenous (i.v.) administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP),increased plasma PRL (Lu, et al., 1973). Quipazine, a 5-HT receptor agonist, also was shown to elicit a rise in PRL (Clemens et al., 1977).

Early work demonstrated that the opiates, most notably MOR, increased PRL release (Meites, 1962; DeWied et al., 1974). Upon the discovery of the endogenous opioid peptides in the brain and hypothalamus, it was anticipated that these peptides would mimic the PRL releasing action of MOR. Indeed, injections of β-END (Rivier et al., 1977), met-enkephalin (MET-ENK) (Bruni et al., 1977), leu-enkephalin (LEU-ENK), and dynorphin (Van Vugt et al., 1981), all have been shown to increase PRL release. In vitro assays have demonstrated that the PRL releasing action of endogenous opioid peptides is via hypothalamic mechanisms, and not directly at the anterior pituitary (AP) (Shaar, 1977; Rivier, 1977). Naloxone, an opiate receptor blocker, was shown to lower basal PRL levels (Bruni et al., 1977; Grandison and Guidotti, 1977; Shaar et al., 1977). These data suggest a possible role for endogenous opioid peptides in tonic control of PRL secretion. Naloxone also decreases the plasma PRL response to ether, heat, and immobilization stress (Van Vugt et al., 1977; Grandison and Guidotti, 1977), and inhibits PRL release induced by the suckling stimulus (Miki et al., 1981).

The endogenous opiate peptides may mediate their action on PRL release by neurotransmitters which are known to regulate PRL release. β-END has been shown to decrease dopamine turnover in the

hypothalamus (Van Vugt et al., 1979; Van Loon et al., 1980). Spampinato et al. (1979) also reported stimulatory serotonergic involvement in the opiate-induced rise in PRL release. There is an increase in 5-HT concentration in the brain and the hypothalamus following β-END injection (Van Loon et al., 1978). Evidence also indicates
that MOR may increase PRL by decreasing cholinergic activity (Fanjul et al., 1981; Shaar and Clemens, 1980). Noradrenergic stimulation appears not be be involved in opiate action on PRL release (Shaar and Clemens, 1980).

Cholinergic drugs have also been shown to inhibit PRL release. Cholinergic agonists, such as pilocarpine or physostigmine, inhibit PRL release in rats, and this effect can be blocked by anti-cholinergic drugs. This action of cholinergic drugs also can be blocked by dopamine depletors (Grandison and Meites, 1976), indicating that cholinergic drugs inhibit PRL by stimulating dopamine synthesis or release. A direct effect of cholinergic drugs on pituitary PRL release also is possible (Fanjul, Galarreta, and Meites, unpublished).

B. Action of Glucocorticoids on PRL Secretion

It has been clearly documented that the glucocorticoids produced by the adrenal cortex inhibit PRL release from the pituitary. Injection of dexamethasone, a synthetic glucocorticoid, inhibited basal PRL release in both rats (Euker et al., 1975) and man (Dussault, 1974). Dexamethasone also inhibited the rise in plasma PRL induced by stress (Harms et al., 1975), or by injection of TRH

(Schwinn, et al., 1976). Removal of glucocorticoid inhibition by adrenalectomy resulted in an increase in basal plasma PRL (Ben-David et al., 1971). Possible mechanisms by which glucocorticoids could depress PRL secretion include: (a) inhibition directly on the pituitary, or (b) modification of neurotransmitter activity in the hypothalamus. Evidence that suggests an action of glucocorticoids on the pituitary include in vitro experiments demonstrating that glucocorticoids can inhibit the spontaneous release of PRL by pituitaries in culture (Leung et al., 1980). Also, corticosterone injected into hypophysectomized rats bearing pituitary grafts underneath the kidney capsule significantly decreased plasma PRL levels (Leung et al., 1980). These results indicate that glucocorticoids may act directly on the pituitary lactotrophs.

There is also evidence that the action of glucocorticoids on prolactin may be mediated by alteration in brain neurotransmitters. After adrenalectomy, brain 5-HT turnover correlates inversely with plasma ACTH during the triphasic response to ADX in rats. That is, 5-HT turnover in the anterior hypothalamus falls abruptly, then returns to normal, and finally decreases again (Van Loon et al., 1982). This decrease in 5-HT turnover may result in receptor supersensitivity causing an increase in serum PRL values. There also is an increase in norepinephrine turnover in the hypothalamus and other brain regions after ADX (Hökfelt and Fuxe, 1972). These results indicate that there are several pathways by which the glucocorticoids can inhibit PRL, including direct action on the pituitary and on the hypothalamus.

C. Control of Glucocorticoid Secretion

It has been recognized for some time that the pituitary regulates corticosterone release from the adrenal gland. Hypophysectomy results in adrenal atrophy and a decrease in plasma corticosterone in rats. ACTH was later identified as the pitiutary hormone responsible for stimulation of adrenal cortical function (Li et al., 1955). ACTH stimulates the release of corticosterone from the adrenal cortex. Corticosterone in turn can act at the hypothalamus or pituitary or both to decrease ACTH secretion in the classical feedback loop. Adrenalectomy results in a rapid decline in blood corticosterone levels. ACTH displays a "triphasic" response to ADX (Dallman et al., 1972). ACTH first rises quickly after ADX, peaking in about 2 hours; by 20 hours, ACTH has decreased to near normal levels; by 96 hours, ACTH rises again and remains elevated thereafter. The effects of neurotransmitters on ACTH release are controversial (see review, Weiner and Ganong, 1978).

D. Effect of Opiates on the ACTH/Adrenal Cortical System

The action of opiates on the hypothalamic-pituitary-adrenal axis is complex. Acute injection of MOR or β-END increases ACTH and corticosterone release (George, 1971; Haracz et al., 1981). However, the ACTH rise induced by various stimuli such as vasopressin and sham-adrenalectomy can be blocked by acute MOR administration (Ohler and Sevy,1956). Chronic administration of MOR results in a decrease of the MOR-induced ACTH rise, but the blockade of the stress-induced release of ACTH by MOR remains intact (Kokka et al., 1973). Naloxone blocks the MOR and β-END induced rise in

ACTH release (Kokka et al., 1973), but NAL alone elicits an increase in plasma ACTH in both rats (Eisenberg, 1980), and in man (Volavka et al., 1979) when administered at doses of 10 mg/kg or above.

ACTH and β-END are derived from a common precursor molecule, pro-opiocortin (Mains et al., 1977), and are released simultaneously in response to stressful stimuli (Guillemin et al., 1977). Therefore, as ACTH secretion increases following ADX, it was also expected that β-END secretion would increase. This was confirmed by Akil et al. (1979) and others who reported elevated plasma β-END and increased pituitary content of β-END after adrenalectomy. It has been postulated that the glucococorticoids act as negative feedback agents for both β-END and ACTH (Giagnoni et al., 1980).

A number of observations have demonstrated an antagonism between opioid peptides and ACTH peptides. For example, injection of $ACTH^{1-24}$ counteracted the rise in plasma PRL elicited by a MET-ENK analog (Ferri et al., 1982), ACTH blocked the analgesic action of MOR (Gispen et al., 1976a; Zimmerman and Krivoy, 1973), and NAL inhibited the $ACTH^{1-24}$ induced increase in grooming behavior (Gispen et al., 1976b). Therefore, it is probable that the opposing actions of glucocorticoids and endogenous opiates interact to influence the secretion of PRL also. This antagonism between opiates and ACTH peptides may be mediated by blockade of opiate receptors by ACTH. The interaction of ACTH fragments and opioid receptors has been demonstrated by displacement of dihydromorphine from opiate receptors by ACTH fragments in vitro. A study of the stereochemical structure of ACTH and β-END has demonstrated configurational similarities, and suggests a molecular basis for the interaction of

ACTH and opioid receptors (Snell and Snell, 1981).

Numerous reports have cited interactions between the endogenous opiates and the adrenal cortical system. The endogenous opioid peptides control PRL release via hypothalamic neurotransmitters. The glucocorticoids appear to influence PRL release both at the pituitary and through the neurotransmitters. The opiates can cause both an increase in plasma ACTH levels and block the stress-induced ACTH release. Finally, it has been reported that ACTH peptides can occupy opiate receptors and block the action of endogenous opioid peptides. The purpose of this study was to investigate the possibility that the endogenous opiate peptides and the glucocorticoids interact in the control of PRL release. In the first experiment, the effect of morphine on plasma prolactin in adrenalectomized rats was compared to the effect of morphine in normal intact rats. Secondly, the effect of length of time after adrenalectomy on the morphine-induced rise and naloxone-induced suppression of plasma prolactin was investigated. To confirm the results of these two experiments, the effect of morphine and naloxone on prolactin in adrenalectomized rats with corticosterone replacement was examined.

II. MATERIALS AND METHODS

A. Animals

Male Sprague-Dawley rats (250-450 g) obtained from Charles River Breeding Laboratory (Wilmington, MA) were housed in a temperature (22°C) and light controlled room (L:D 14:10, lights on at 0500 h). Food (Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO) and water were available ad libitum.

B. Surgery

Animals were bilaterally adrenalectomized or sham operated via a lateral incision under ether anesthesia. Adrenalectomized animals received 0.9% NaCl drinking solution, and the diet was supplemented with sugar cubes to help maintain normal blood sugar and electrolyte levels. Four to six days prior to the experiments, a silastic indwelling atrial cannula was implanted into each rat via the right external jugular vein under ether anesthesia. The distal end of the cannula was routed s.c., and exited 1 cm posterior to the base of the skull. The tubing was filled with heparinized saline (100 I.U./ml) and tied. All rats received an I.M. injection (0.3 ml) of benzathine-procaine penicillin G at the time of surgery to prevent infection. After cannulation, rats were housed in individual cages (18x18x24 cm).

C. Pharmacological Agents

Morphine sulfate (Mallinckrodt, Inc., St. Louis, MO), and naloxone HCl (Endo Laboratories, Garden City, NY), were dissolved in 0.87% sterile saline (0.15 M NaCl) prior to injection.

D. Experimental Procedure

Animals were acclimated to the experimental room for a period of at least three hours for one or two days immediately prior to the experiments. On the day of the experiment, rats were brought to the experimental room and 20 cm extensions of silastic tubing were attached to the cannula and threaded through the top of the cage. After removal of the void volume (0.2 cm), blood samples of 0.8 to 1 ml were collected into heparinized syringes. Plasma was separated by centrifugation, decanted and frozen on dry ice. Blood cells were resuspended in sterile saline and reinjected throughout the experiment to maintain blood volume. Plasma samples were stored at -20° C until assayed for PRL and corticosterone. All drugs and control solutions were injected i.v. via the cannula.

E. Radioimmunoassay of Prolactin and Corticosterone

Plasma PRL concentration was measured with materials provided by Dr. A. Parlow (NIADDK, Bethesda, MD). Anti-rat prolactin antibody was a gift of Dr. David Chen. Rat PRL was radiolabelled using a chloramine-T method (Greenwood et al., 1963) and chromatographed on a Sephacryl S-200 column (Pharmacia Fine Chemicals, Piscataway, NJ). Only those volumes which gave hormone values which corresponded to the linear portion of the standard were used. Data are expressed in terms of NIH-PRL RP-1. The minimum detectable dose was 0.09 ng/tube and 50% inhibition of tracer binding was 0.62 ng/tube. The intra- and interassay coefficients of variation (n=8) were 6.5% and 8.2%, respectively.

The corticosterone assay is based on a method described by

Gomez-Sanchez et al. (1975). Anti-rat corticosterone antibody was a generous gift from Dr. Gordon Niswender (Colorado State Univ., Ft. Collins, CO). Dextran-coated charcoal was used to separate bound from free tracer. The minimum detectable dose was 6 pg/tube and 50% inhibition of tracer binding was 200 pg/tube. The intra- and interassay coefficients of variation (n=4) were 5.9% and 41%, respectively.

F. Statistics

An RIA program utilizing the log-logit method was used to calculate plasma PRL and corticosterone values. Data were analyzed using the BMDP2V computer program for analysis of variance including repeated measures (Health Science Computing Facility, Univ. of Calif., Los Angeles, CA). Subsequent tests were performed using the Newman-Keuls' procedure (Winer, 1971).

III. EXPERIMENTAL

A. Experiment 1: The Effect of Morphine on PRL Release in Adrenalectomized Male Rats

1. Objective

The adrenal steroids and the endogenous opioid peptides oppose each other in their action on PRL release. The opiates stimulate PRL release, whereas the glucocorticoids inhibit PRL release. If the opiates and glucocorticoids interact to control PRL, then removal of one of the components (glucocorticoids) would be expected to produce an aberrance in the normal response to the second component (MOR). Therefore, the response of PRL to MOR administration in adrenalectomized rats was investigated.

2. Materials and Methods

Two groups of rats, intact and twenty day adrenalectomized rats, were injected with morphine at 3 mg/kg, IV. Blood samples were collected at -40, -20, 10, 30, 50, 70, and 90 min via the cannula. Plasma was separated and frozen.

3. Results and Conclusions

Results are presented in Figure 1. Baseline PRL levels were elevated to near 40 ng/ml in adrenalectomized rats, as compared to 13 ng/ml in control rats. Injection of MOR significantly increased plasma PRL by 10 min after administration in control rats. Prolactin values quickly fell to baseline by 30 min. In adrenalectomized rats, MOR also caused a significant rise in plasma PRL which peaked at 10 min after injection, and was significantly greater ($p<0.05$) than the peak response in control rats. The control rats exhibited a 216% increase; the adrenalectomized rats

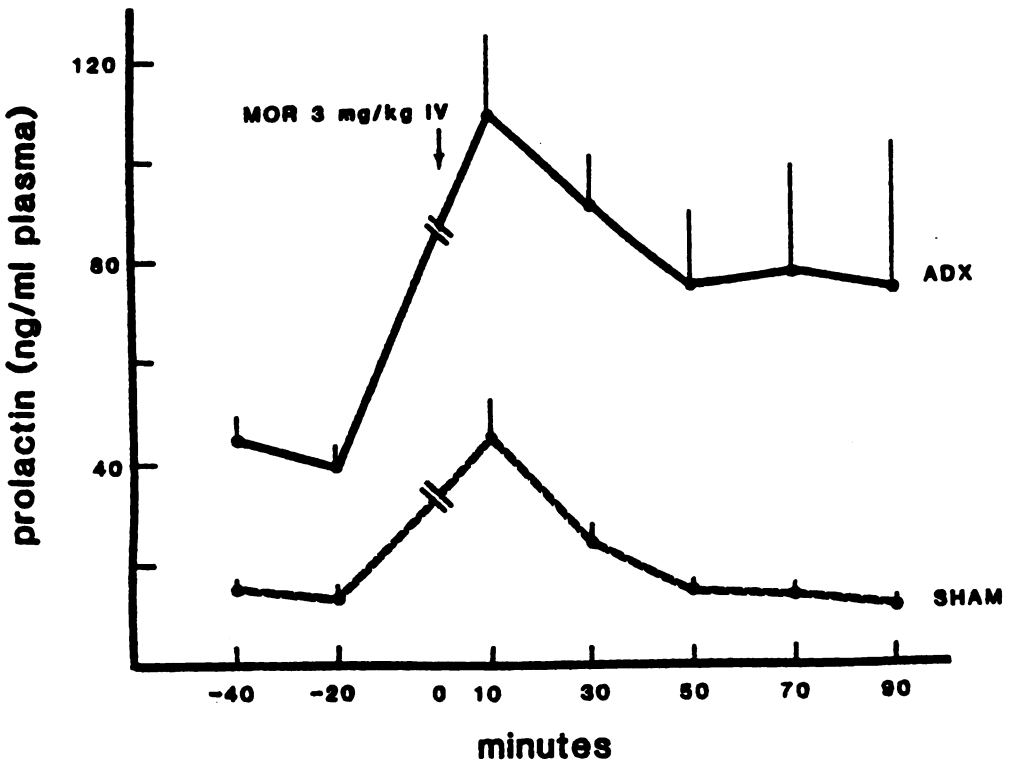

Figure 1. Plasma prolactin (ng/ml) in adrenalectomized and sham-operated male rats before and after injection of morphine (3mg/kg,IV). Each point represents the mean ± SEM.

exhibited a 255% rise. In contrast to controls, PRL in ADX rats remained elevated throughout the 90 min sampling time and remained significantly elevated at 30, 50, and 70 minutes.

These results demonstrate an enhanced response of PRL release to MOR in the absence of the PRL suppressing action of corticosterone. This suggests that the glucocorticoids can modulate the PRL response to opiate stimulation.

B. Experiment 2: The Effect of Morphine and Naloxone on PRL Release in Adrenalectomized Rats Over Time

1. Objective

ACTH levels fluctuate for the first few days after ADX in a 'triphasic response' manner as explained above in Section I.C. ACTH and β-END are released together from the pituitary, and it is therefore possible that the plasma β-END levels mimic the 'triphasic response' of ACTH.. It was of interest to see if the proposed fluctuations of endogenous opiate levels would affect the response of PRL to MOR in adrenalectomized rats. The initial experiment was repeated using rats at different times after ADX.

2. Materials and Methods

Eighty male Sprague-Dawley rats were adrenalectomized on the same day. Cannulae were implanted 4-5 days before the experiment. Experiments were performed 1, 3, 5, 10 or 20 days after adrenalectomy, or 6 days following sham-adrenalectomy. Rats in each experiment received an i.v. injection of MOR (3 mg/kg), NAL (1 mg/kg), or saline (0.1 mg/kg). Blood samples of 1 ml were collected via cannula.

3. Results

Baseline plasma PRL values were increased above sham-control basal values at all time points after ADX (Figure 2). Morphine injection produced a significant rise in plasma PRL in all groups. The peak PRL value on days 1,3, and 5 after ADX were over 100 ng/ml as compared to 42 ng/ml in controls. As in Experiment 1, plasma PRL remained elevated in adrenalectomized rats throughout the 60 min sampling period, whereas in controls PRL values returned to baseline values by 60 min. Injection of NAL into sham-controls decreased basal PRL. However, after adrenalectomy NAL did not suppress basal PRL. From this profile of responses over time 5 days post-ADX rats were chosen as the model to be used in future work. By five days after ADX blood and brain levels of ACTH have stabilized (Van Dijk et al., 1981; Van Loon et al., 1981). Also, physiological adaptation to adrenalectomy has occured, diminishing the stress-induced rise of PRL as seen in the saline injected group on day 1.

C. Experiment 3: Effect of Corticosterone Replacement on MOR-induced Rise of Prolactin in Adrenalectomized Rats

1. Objective

(a) The purpose of this experiment was to see if the MOR-induced rise in PRL release in adrenalectomized rats can be returned to normal by replacement with corticosterone. If the enhanced morphine-induced rise in prolactin in adrenalectomized rats is due to the loss of inhibitory action by glucocorticoids, then replacement with corticosterone should attenuate the prolactin response to morphine to the same level as the controls.

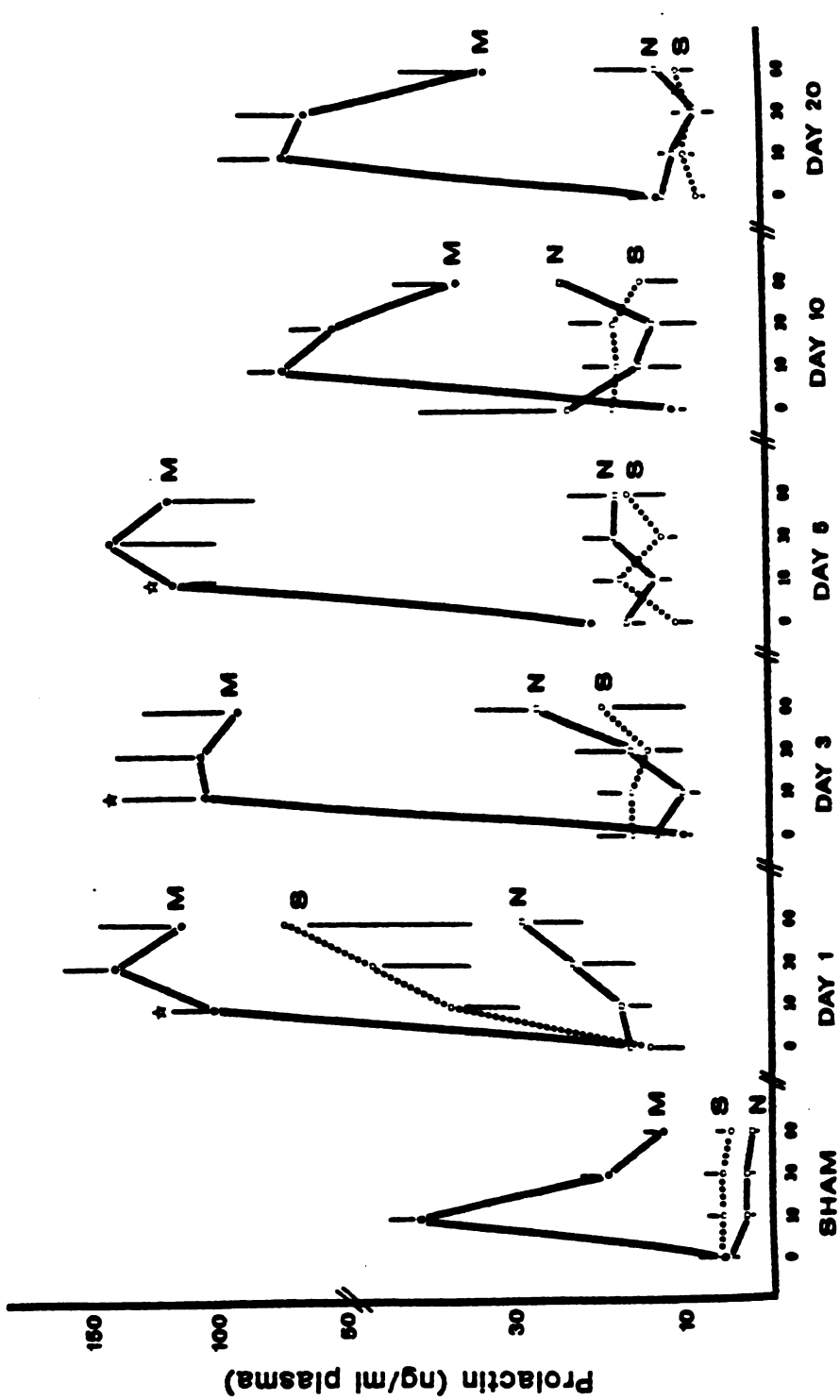

Figure 2. Plasma prolactin (ng/ml) after drug injection on varying days after adrenalectomy. M=morphine (3mg/kg,IV). N=naloxone (1 mg/kg,IV). S=saline (1 ml/kg,IV). Each point represents the mean ± SEM. Drug injection is at time=0. Blood was sampled immediately prior to injection and 10, 30, and 60 minutes after injection.

(b) To see if NAL will function as an opiate antagonist in adrenalectomized rats by blocking the MOR-induced rise of PRL.

2. Materials and Methods

At the time of ADX or sham-ADX, rats were given s.c. implants of either a 50% corticosterone pellet or vehicle (cholesterol) pellet. Corticosterone pellets were made according to the procedure described by Meyer et al. (1979). To achieve a 50% corticosterone pellet, corticosterone (Sigma Chemical Co., St. Louis, MO) was mixed with a equal weight of cholesterol (Sigma Chemical Co.), melted, and transferred to a paraffin mold. Pure cholesterol pellets were used as placebos in control rats. The final weight of the finished pellets was adjusted to 100 mg. Serum corticosterone values after implantation of 50% and 100% pellets in adrenalectomized rats were measured by RIA. Five days after adrenalectomy, 3 groups of rats (N=8) were injected with MOR (3 mg/kg i.v.). A fourth group of adrenalectomized rats (N=7) received a simultaneous injection of morphine (3 mg/kg) and naloxone (1 mg/kg).

3. Results

The effects of replacement of corticosterone in adrenalectomized rats is shown in Figure 3. Prolactin in adrenalectomized rats rose from 19.1 $\pm$ 6.2 ng/ml to 124.6 $\pm$ 12 by ten minutes, remained elevated at thirty minutes and declined thereafter. Plasma prolactin in adrenalectomized rats with costicosterone implants rose from 14.2 $\pm$ 3.7 to 78.0 $\pm$ 1.3 and returned to baseline by 30 minutes. The peak prolactin value in the adrenalectomized rats with corticosterone implants is significantly ($p<0.05$) different from adrenalectomy only rats. The plasma

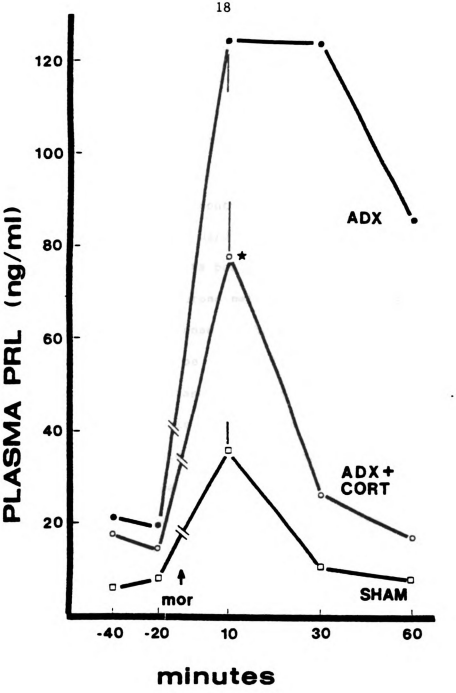

Figure 3. The effect of morphine (3mg/kg,IV) on plasma prolactin values (ng/ml) in adrenalectomized rats and adrenalectomized rats bearing corticosterone pellets. Each point represents the mean ± SEM. *, significantly different from ADX and Sham values at 10 minutes.

prolactin values rose 556% from baseline as compared to a rise of only 368% in sham-operated rats. Prolactin in corticosterone-replaced adrenalectomized rats rose 450%. Corticosterone replacement abolished the prolonged elevation of prolactin in adrenalectomized rats and prolactin was returned to baseline at 30 minutes as in control rats. Plasma corticosterone concentration was 21.6 $\pm$ 3.7 ug/dl in control animals and 9.6 $\pm$ 1.8 ug/dl in adrenalectomized rats bearing corticosterone pellets. A more concentrated corticosterone pellet would be expected to further decrease the prolactin response to that of intact rats and 100% corticosterone pellet will be used in future work. As seen in Table 1, naloxone injected simultaneously with morphine completely blocked the morphine-induced rise in prolactin in adrenalectomized rats.

These results indicate that the loss of corticosterone is responsible at least in part for the larger than normal and prolonged morphine-induced rise in prolactin in adrenalectomized rats. Naloxone does act as an opiate agonist to block the morphine-induced rise in prolactin in adrenalectomized rats in spite of its inability to lower basal plasma prolactin in adrenalectomized rats.

Table 1. Plasma prolactin values in adrenalectomized rats before and after drug treatment.

	Plasma prolactin (ng/ml)				
	minutes				
	-40	-20	10	30	60
MOR	21.1±4.6	19.1±6.2	124±12	123±21	85.6±23
MOR + NAL	30.2±11	30.6±12	24.0±5	11.6±2.1	11.0±2.7

Values are means ± SEM. N = 7 or 8. Drug injection is at time equals 0.

D. Experiment 4: Effect of Corticosterone Replacement on Naloxone-induced Suppression of Prolactin in Adrenalectomized Rats

1. Objective

The purpose was to see if corticosterone replacement can reinstate the naloxone-induced suppression of basal prolactin that is not observed in adrenalectomized rats.

2. Materials and Methods

At the time of adrenalectomy or sham-adrenalectomy, the rats received a subcutaneous implant of a 100% corticosterone pellet or vehicle (cholesterol) pellet. Five days after adrenalectomy, drug injection of either naloxone at 1 mg/kg, IV or saline at 1ml/kg, IV were given and blood samples were collected by cannula.

3. Results

The results (Figure 4) show that both the sham rats and the adrenalectomized rats with corticosterone replacement exhibited a naloxone-induced suppression of basal prolactin values. Plasma prolactin in sham rats fell from 3.8 $\pm$ 0.5 ng/ml to 1.9 $\pm$ 0.2 ng/ml. Plasma prolactin in adrenalectomized rats with pellets fell from 4.6 $\pm$ 1.2 to 2.2 $\pm$ 0.5. As before, plasma prolactin in adrenalectomized rats receiving naloxone was not different from plasma prolactin in adrenalectomized rats receiving saline injections.

Plasma corticosterone values in adrenalectomized rats with pellets was 13.9 $\pm$ 1.4 ug/dl and in control rats were 12.2 $\pm$ 4.6 ug/dl.

The results demonstrate that the replacement of corticosterone in adrenalectomized rats returns the naloxone-induced suppression of

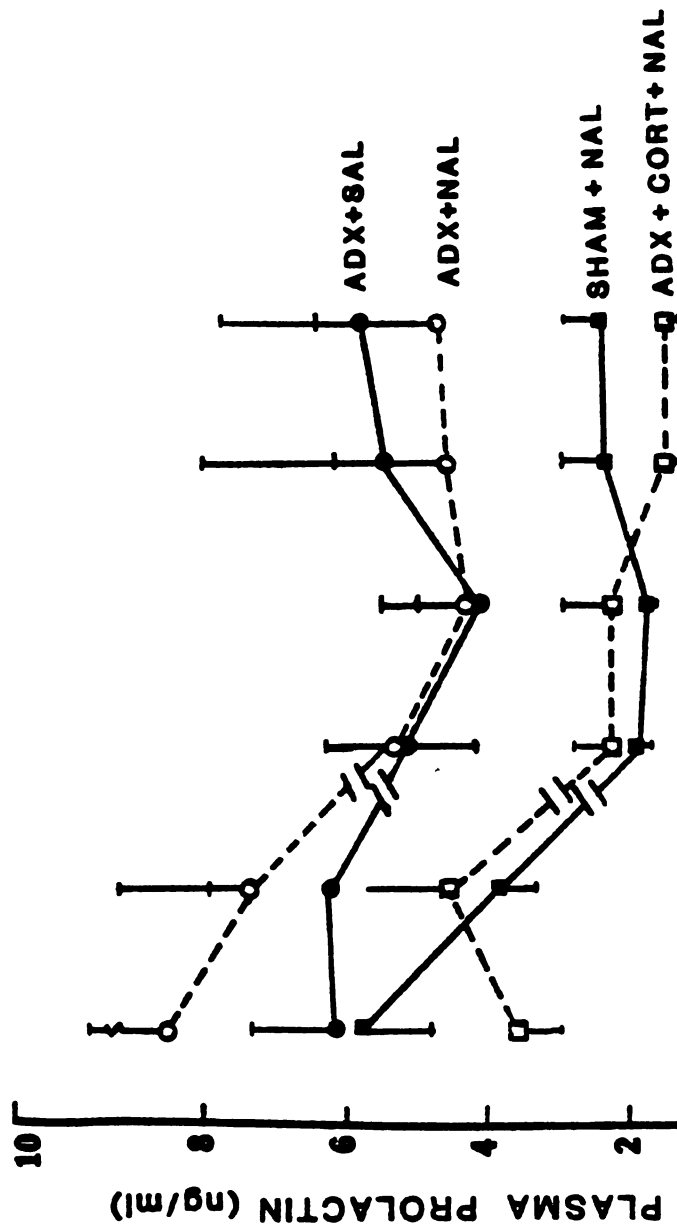

Figure 4. Effect of naloxone (1 mg/kg,IV) on plasma prolactin values (ng/ml) in adrenalectomized rats and adrenalectomized rats bearing corticosterone pellets. Each point (n = 8) represents the mean ± SEM.

basal prolactin to normal.

IV. GENERAL DISCUSSION

The data presented in this thesis indicate that the adrenal steroids can influence the opiate-induced release of PRL. Basal plasma PRL concentrations in ADX rats were significantly greater than in sham-controls after MOR administration. The triphasic fluctuations of ACTH and possible fluctuations of β-END do not appear to influence the MOR-induced stimulation of PRL release. Naloxone suppressed basal PRL in control rats but not in adrenalectomized rats. Naloxone is able to block the MOR-induced rise in PRL in adrenalectomized rats. Replacement of corticosterone significantly reduced the morphine-induced rise in prolactin observed in adrenalectomized rats. Likewise, corticosterone replacement in adrenalectomized rats reinstated the NAL-suppression of basal PRL.

The opiates have been reported to increase PRL by acting through the neurotransmitters dopamine and serotonin. Dopaminergic agonists block MOR-induced PRL release (Van Vugt et al., 1979). Therefore, a decrease in DA turnover would increase prolactin. A change in dopamine turnover after ADX could account for an alteration of the PRL response to MOR. However, no change in DA content or metabolism after ADX in median eminence, medial basal hypothalamus or pituitary has been found (Fuxe et al., 1973; Leung et al., 1980).

One mechanism by which the opiates appear to increase prolactin is by increasing serotonin turnover. Serotonin turnover in the hypothalamus is increased by β-END injection (Van Loon and DeSouza, 1978). Spampinato et al. (1979) and Koenig et al. (1979)

were able to block the met-enkephalin-induced rise of PRL with 5-HT antagonists, but others were not able to block the MET-ENK-induced PRL rise by PCPA synthesis inhibition of 5-HT (Cusan et al., 1977). A change in 5-HT turnover following adrenalectomy could account for an increase of the prolactin response to morphine. Although there are some conflicting reports (Telegdy and Vermes, 1975), investigators have found an increase in 5-HT concentration in the medial basal hypothalamus (Leung et al., 1980) and increase in 5-HT content in the dorsal hippocampus (Rotsztejn et al., 1977) following adrenalectomy. Van Loon et al. (1982) has reported a decrease in 5-HT turnover and synthesis rate in the anterior hypothalamus which correlates inversely with the triphasic changes of ACTH after ADX. Perhaps the decrease in 5-HT turnover results in a receptor supersensitivity. Injection of the serotonin agonist, fluoxetine, or antagonist, cyproheptadine, did not alter plasma PRL levels in intact rats, but fluoxetine increased and cyproheptidine suppressed PRL in ADX rats (Leung et al., 1980). Thus, 5-HT may participate actively in the rise of PRL after ADX. There is evidence that MOR is metabolized more slowly in adrenalectomized than in intact rats (Holaday et al., 1979). A decrease in metabolism rate could contribute to the prolonged PRL rise and higher peak response in adrenalectomized rats.

Naloxone consistently depresses basal PRL in intact rats (Bruni et al., 1977). However, NAL failed to suppress PRL in adrenalectomized rats. This suggests that adrenalectomy disrupts the tonic stimulatory input of endogenous opioid peptides. These results are in disagreement with those by Siegel et al. (1982) who

reported suppression of basal PRL by NAL in ADX rats. This discrepancy may be due in part to differences in experimental procedures. In the work of Siegel et al. (1982), naloxone was injected i.p. and this regiment of handling and injecting animals may have caused a stress-induced rise in PRL. Also, blood was sampled by decapitation and therefore animals did not serve as their own control as in this work wherein NAL was injected iv and blood sampled by cannula.

Naloxone is known to act as an opiate agonist at high doses, but this has been demonstrated mostly with regard to behavioral effects of NAL (Sawynok et al., 1979). The one case in which NAL has been reported to act as an agonist in an endocrine system involves the adrenal cortical system. Acute injection of MOR increased glucocorticoids (Nikodijevic and Maickel, 1967) and paradoxically, high doses of NAL also increased glucocorticoids both in man (Volvaka et al., 1979) and rats (Eisenberg, 1980). A decreased metabolism of NAL in adrenalectomized rats may yield an effectively higher dose resulting in an agonistic instead of an antagonistic action. Another possible explanation for the lack of a NAL-induced suppression of PRL in ADX rats is that corticosterone may be required for NAL to suppress PRL. MacLennan et al. (1982) reported that corticosterone is required for opiate-induced analgesia. Thus it may be that corticosterone modulates the opiate control of PRL release.

Further work is needed to delineate the interactions between corticosterone and opiate control of prolactin secretion. The effects of stress could be removed by in vitro work such as

co-incubation of hypothalamic and pituitary tissue. This would also remove the potentially confounding problem of effects of higher than normal plasma end on prolactin release. Other possible mechanisms include alterations in neurotransmitter concentration and turnover or changes in the number or sensitivity of receptors for opiates or corticosterone.

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