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Dual Role of Glucocorticoids in Suckling-Induced Prolactin Secretion

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The exact contribution of corticosteroids to the control of prolactin secretion in lactating rats is poorly understood. Therefore, the present studies were focused on the effect of adrenalectomy and dexamethasone treatment on the suckling-induced prolactin release. Animals were adrenalectomized on the 3rd day of lactation and tested on the 7th day of lactation. In adrenalectomized animals, the suckling stimulus failed to induce the characteristic increase in plasma prolactin levels. Dexamethasone pretreatment (400 µg/kg b.w. s.c. 24, 48, 72 h before testing) of adrenalectomized rats restored this prolactin response. The same treatment with dexamethasone given to control animals attenuated the suckling stimulus induced prolactin response. The present findings indicate that corticosteroids are essential for a basic prolactin response of lactating rats.

Key Words: Prolactin; adrenalectomy; corticosteroids; dexamethasone; suckling stimulus; lactation.

Introduction

It is well known that prolactin (PRL) is the essential hormone for milk production *(1,2)*. Several reports indicate that adenohypophysial PRL secretion is altered in the absence of corticosteroids. In non-lactating rats, plasma levels of PRL increase significantly after adrenalectomy, and this effect of adrenalectomy can be reversed by administration of corticosteroids *(3–6)*. Adrenalectomized (ADX) dams have consistently high levels of plasma PRL throughout the entire lactation period *(7)*. These findings indicate a tonic inhibitory influence of corticosteroids on PRL secretion.

Some years ago, we observed *(8)* that in lactating rats united with their pups, plasma PRL levels decreased significantly after exposure to ether vapor or injection of formalin under the skin. Administration of formalin to ADX dams united with their litter caused a slight, immediate decrease, followed by a transitory elevation and a subsequent small second decrease in blood PRL concentration. This observation indicated that in ADX rats, the PRL response to formalin stress is modified. In the present studies, the PRL response to another stimulus was examined in ADX dams, i.e., we were interested in the PRL releasing effect of the suckling stimulus in ADX lactating rats. It is well known that the suckling stimulus causes, via an ascending sensory neural pathway, a rapid rise in plasma PRL levels *(9–11)*, and in contrast, separation of dams from the litters results in an immediate marked inhibition of PRL release *(12)*.

In the present study, ADX dams were untreated or treated with dexamethasone (DEX), a synthetic glucocorticoid agonist, and exposed to the suckling stimulus to determine the role of glucocorticoids on suckling–induced PRL secretion.

Results

In animals in which the adrenals had been removed, the suckling stimulus failed to induce an increase in plasma PRL levels (Fig. 1). In such dams, there was absolutely no elevation in plasma PRL within 60 min after the pups were replaced and they suckled. DEX treatment of ADX rats restored the suckling-induced PRL response. In DEX-treated controls receiving DEX 24, 48, and 72 h before testing, there was a very attenuated PRL response to the suckling stimulus. It is noteworthy that before separation, the PRL levels are higher in the two ADX groups compared to the two groups with adrenals. This difference is statistically significant ($p < 0.05$).

There was no difference in the behavior of the mothers or pups of the four groups after the pups were replaced. Pups of each group (litters were frequently changed among groups, see Materials and Methods) started suckling within a few minutes after replacement.

Discussion

Our present finding shows that removal of the adrenals interferes with a basic pituitary PRL response induced by the suckling stimulus. It should be emphasized that we observed in our present studies that in ADX rats, a physiological neural stimulus is not able to induce rapid PRL release.

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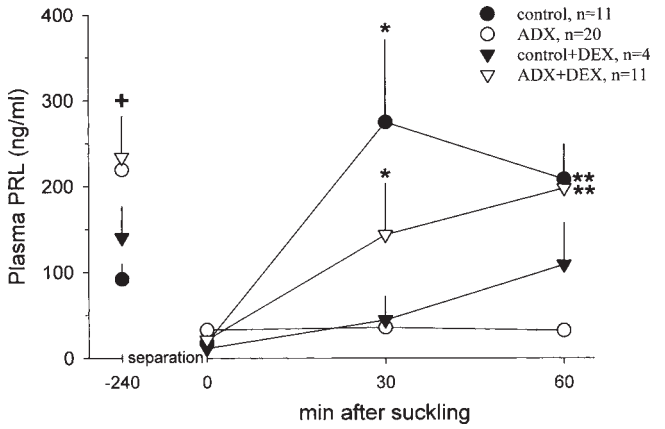

Fig. 1. Effect of adrenalectomy (ADX) and/or dexamethasone (DEX) treatment on PRL responses induced by the suckling stimulus. First blood samples were taken at 09:00 a.m., and then pups were removed from their mothers. Four hours later, blood samples were taken again and pups were reunited with the mother for suckling. Group averages (±S.E.M.) are depicted. $^{+}p < 0.05$ indicates both ADX and DEX treatment effects prior to separation (2-way ANOVA). $*p < 0.05$, $**p < 0.01$ compared to the 0 min values (Paired-samples T-test). The difference in the time course of the hormone changes between the control and the ADX group as well as between the ADX and the ADX plus DEX group is statistically significant ($p < 0.05$).

This finding does not mean that such animals are not able to secrete PRL. On the contrary, it has been previously pointed out that ADX lactating rats have elevated plasma PRL levels *(7)*. It appears that adrenalectomy interferes with a neuroendocrine reflex response, i.e., with an acute PRL response to a neural stimulus, but not with the basic mechanism of PRL secretion. Our finding is somewhat intriguing when compared with observations obtained by van der Schoot and de Greef *(7)*. These authors have previously investigated the PRL response to suckling in ADX rats. In those repeatedly cited studies, the authors found that during the second half of lactation, adrenalectomy can prevent the gradual decrease of PRL response induced by suckling detected in intact mothers. The plasma PRL levels of these animals were significantly higher 1 h after stimulus and remained high 2–3 h after replacement of the pups. It must be emphasized that their experimental designs were quite different from ours. In their experiments, lactating rats were not only ADX but also ovariectomized (OVX). The combined surgery was made on the day of parturation, and the animals were tested on day 13 or 20 of lactation. Moreover, litters were replaced with mothers after 6 h separation, and only hourly blood samples were taken for 3 h. In contrast, animals in our experiment were ADX only (on day 3 of lactation) and exposed to the suckling stimulus on day 7 of lactation. Blood samples were taken prior to separation, and at 30 and 60 min intervals during the first hour of the suckling stimulus following 4 h separation. Taya and Sasamoto *(13)* have noted that plasma PRL levels are not the same in ADX and ADX plus OVX rats. In ADX rats, the high levels of the hormone in the blood decrease by day 20 of lactation, while in the case of combined surgery, this decrease happens days earlier. Thus, it appears that the findings of van der Schoot and de Greef and our observations are only slightly related.

Literature data on the stimulatory action of corticosteroids on PRL secretion are rather indirect. It is well known that adrenalectomy reduces milk production, and administration of corticosteroids to ADX animals restores lactation *(14)*. Our present finding that the PRL response to the suckling stimulus is missing in ADX rats, and this response can be restored by DEX, is the first demonstration that corticosteroids are required for this basic pituitary PRL response of lactating rats. Our observations do not provide any information on the site and mechanism of action of DEX. Theoretically, it could be exerted either at the level of the pituitary gland, by sensitizing the mammotropes to PRL releasing substances, or at the level of the CNS. In this latter case, the influence is probably mediated via the hypothalamus, presumably involving PRL-releasing peptides such as thyrotropin-releasing hormone, vasoactive intestinal peptide, oxytocin and the like *(1)*.

Interestingly, Weidenfeld and Feldman *(15)* have recently reported that the serum ACTH responses to neural stimuli (both photic and acoustic stimuli), tested at the same times, were completely inhibited after adrenalectomy. In contrast, administration of either insulin or 2-deoxyglucose caused a marked increase in serum ACTH levels. In ADX rats, implanted with corticosterone pellets, which produced basal serum levels of corticosterone, median eminence corticotropin-releasing hormone-41 levels and serum ACTH were similar to control sham-operated animals. Exposure to both neural stimuli resulted in a significant depletion in corticotropin-releasing hormone-41 content of the median eminence and a rise in serum ACTH as in the respective controls. Thus, it appears that corticosteroids are required for both PRL and ACTH response of the pituitary gland to neural stimuli. In the absence of these hormones, neural stimuli are ineffective in inducing PRL or ACTH release.

We found that suckling induced PRL response was attenuated in intact rats treated with DEX. This finding is in line with several observations indicating an inhibitory influence of corticosteroids on PRL secretion: (i) ADX rats have elevated plasma PRL levels [*(3–6,8)* and present finding, Fig. 1]; (ii) there is no decrease in plasma PRL in ADX animals at late lactation *(7)*; (iii) DEX, when given on the day of testing, inhibits suckling-induced PRL release *(16)*. The site and mode of inhibitory action of adrenocortical hormones on PRL secretion is ill-defined. Literature data indicate that the inhibitory effects of the corticosteroids may be exerted through actions at the anterior pituitary gland and/or at the central nervous system, notably the hypothalamus. It is known that at the pituitary level, glucocorticoids inhibit both the synthesis *(17)* and the release of PRL *(18,*

19). Using an in vitro model to examine the requirement for protein synthesis, Taylor et al. *(20)* have shown that the inhibitory actions of DEX on PRL release are dependent on *de novo* protein synthesis. Moreover, the authors have provided evidence that both lipocortin 1-dependent and lipocortin 1-independent mechanisms are involved in the action of DEX. In addition, glucocorticoids could alter the sensitivity of mammotropes to hypothalamic factors regulating PRL secretion. Concerning the central nervous system site of action, glucocorticoids acting at the level of the hypothalamus could directly alter the activity and sensitivity of the main PRL release inhibiting system, namely the tuberoinfundibular and periventriculohypophyseal dopaminergic system. Tuberoinfundibular neurons, located in the posterior part of the nucleus arcuatus, and periventriculohypophyseal neurons, located in the anterior periventricular region, are well accepted as major physiological regulators of adenohypophyseal PRL secretion *(21)*. Another possibility is that glucocorticoids indirectly influence the function of tuberoinfundibular and periventriculohypophyseal neurons as well as mammotropes via modulation of other inhibitory systems, such as the medial basal GABAergic system, opioid system, or altered secretion of hypothalamic PRL releasing factors *(22–24)*.

We found that DEX was required to restore the suckling induced PRL response of ADX rats. At the same time DEX, when given to intact rats, attenuated the same response. To explain this seeming discrepancy, it has to be taken into account that in the case of DEX treatment of ADX animals, DEX was a corticosteroids replacement, while in the control rats, DEX resulted in a marked, artificial elevation of plasma corticosteroid levels. As mentioned above, high concentrations of corticosteroids in the blood exert an inhibitory influence both on the synthesis *(17)* and the release of PRL *(18,19)*.

In summary, our findings indicate that corticosteroids are required for the suckling-induced release of PRL. Besides this action of corticosteroids, these hormones appear to exert an inhibitory influence on PRL secretion. The latter action is presumably mediated by the hypothalamic dopaminergic systems, which provides a tonic inhibition of PRL release and/or the pituitary gland itself.

Materials and Methods

Primiparous lactating rats of the Sprague-Dawley strain were used. They were caged individually in an air-conditioned room (temperature 21–24 C°) with alternating 14 h light and 10 h darkness. The animals received food and water *ad libitum*. Litter size was reduced to eight on the second day postpartum.

To study the suckling stimulus induced PRL response of ADX and DEX-treated rats, the experiment consisted of the following groups:

- Intact group,
- ADX group,
- DEX-treated intact group,
- DEX-treated ADX group.

All experiments were in accordance with the Guide of the University for the Care and Use of Laboratory Animals.

Adrenalectomy

Dams were ADX under ether anesthesia on day 3 of lactation. These rats had free access to saline. The weight of the litters was measured once a day. To avoid differences in litter weight (which could influence suckling), litters were frequently changed among groups.

Dexamethasone Treatment

The dams received 400 µg/kg body weight dexamethasone phosphate (Oradexon, Organon; Oss, The Netherlands) sc 24, 48, 72h before testing. The other animals were injected with saline.

Blood Sampling

Two days prior to blood sampling, a permanent cannula (polyethylene tubing i.d. 0.50 mm, o.d. 1.00 mm) was implanted under ether anesthesia into the jugular vein allowing frequent blood sampling in undisturbed, freely moving rats. The animals were habituated to the procedure of blood sampling by connecting them to a polyethylene tube every day.

On the 7th day of lactation blood samples were collected before separation (at 09:00 AM) and 4 h after separation (at 01:00 PM). Then the pups were replaced and blood samples were taken 30 and 60 min after replacement of the pups. At each time, 200 µl blood was withdrawn and replaced with saline. The plasma was separated and stored at –20ºC until assayed for PRL.

Hormone Determination

PRL was measured by radioimmunoassay (RIA), with kits kindly provided by NHPP, NIDDK & Dr. A. F. Parlow. The RIA procedure was similar to the instructions supplied with the kit, with previously described modifications *(25)*. We used the Chloramine-T method for iodination and protein A (BactASorb, Human Rt, Gödölló, Hungary) for separation of bound and free hormone. Data collection and calculations for curve fitting were made by the LKB Clinigamma software for PC. The data were expressed in terms of NIAMDD-Rat-RP-3. The within- and between-assay variances were 10% and 14%, respectively.

Statistical Analysis

Statistical analysis of the data was performed with analysis of variance (ANOVA). For the PRL levels in the lactating dams at the beginning of each experiment, we applied two-way ANOVA with between-subject factors ADX and DEX.

To test the effects of the various experimental manipulations on PRL levels, we applied repeated ANOVA measures. To analyze changes in PRL levels within an experimental group, a paired sample T-test was used. Data were expressed as means ± S.E.M. A p value of <0.05 was taken as indicative of statistical significance for the tests.

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