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Effect of Posterior Pituitary Lobectomy on In Vivo and In Vitro Secretion of Prolactin in Lactating Rats

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The effect of removing the posterior and neurointermediate lobes (PLX) of the pituitary gland of lactating rats was determined on both suckling-induced release and transformation of prolactin (PRL), and on regionalization of PRL release. Sixteen hours, or 1 or 4 d after either PLX or sham surgery, acute (15-min) suckling was applied. Also, regionalization of PRL release was analyzed by incubating the central and peripheral regions of APs from nonsuckled rats. Plasma PRL was analyzed by radioimmunoanalysis (RIA), whereas anterior pituitary (AP) PRL content and in vitro released PRL were determined by polyacrylamide gel electrophoresis. Plasma PRL increased 25- to 30-fold after suckling in intact and sham, and 10- to 15-fold in 1- and 4-d PLX rats, but no change occurred on either 16-h PLX nonsuckled and suckled rats. Also, PRL transformation occurred in intact, sham, and 4-d PLX suckled rats, but not in 16-h sham, or in 16-h and 1-d PLX suckled rats. Finally, the higher secretion of PRL shown in vitro by the central region of APs from intact and sham was not observed in APs from PLX rats. These results show that PLX transiently depresses the suckling-regulated PRL transformation and release. Likewise, influences from the posterior and/or neurointermediate lobes may determine regionalization of PRL release.

Key Words: Prolactin; posterior lobectomy; adenopituitary regionalization; PRL transformation.

Introduction

In the present study, we analyzed the effect of removing the posterior and neurointermediate lobes (PNIL) of the pituitary gland of lactating rats on the suckling-induced transformation and release (Grosvenor et al., 1979; Mena et al., 1982, 1984, 1989a, 1992, 1993; Lawson et al., 1987) of prolactin (PRL), and on regionalization of PRL secretion (Boockfor and Frawley, 1987; Mena et al., 1993; Frawley, 1994). The reason for these experiments was that in addition to well-established hypothalamic mechanisms regulating PRL transformation and release, i.e., dopamine (DA) and secretagogues, such as thyrotropin releasing hormone (TRH) (Grosvenor et al., 1984; Mena et al., 1989b), it has been suggested that other PRL secretagogues originated in the PNIL (Murai and Ben-Jonathan, 1987; Hyde and Ben-Jonathan, 1987; Landon et al., 1990), and reaching the anterior pituitary (AP) via the short portal vessels, may physiologically stimulate PRL release and may influence PRL regionalization (*see* Frawley, 1994 for review) by the lactating rat AP. However, it has not been determined which interactions these additional mechanisms may exert with PRL transformation and release, and whether indeed influences from the PNIL may determine PRL regionalization.

In the current model of PRL secretion, i.e., that in which PRL transformation precedes and may determine PRL release (Grosvenor et al., 1979; Mena et al., 1982; Lawson et al., 1987), phasic release of PRL is related with prior DA-regulated PRL transformation (Grosvenor et al., 1980a, 1984; Frawley, 1994), and this is associated with changes in turnover rates in hypothalamic DA (Mena et al., 1976; Plotsky et al., 1982; Grosvenor et al., 1984; Rondeel et al., 1988; Frawley, 1994), and in blood concentrations of DA and TRH within the long portal vessels (de Greef and Visser, 1981; de Greef et al., 1981; Grosvenor et al., 1984; Frawley, 1994). Therefore, it is not clear why selective removal of the posterior and neurointermediate lobes, in which the tuberoinfundibular hypothalamic-pituitary connection is unaffected, completely abolished the suckling-induced release of PRL (Murai and Ben-Jonathan, 1987). Because of this, in the present study we sought to determine how necessary the integrity of the PNIL is for the physiological release of PRL by suckling in the lactating rat, as well as for PRL transformation and for regionalization of PRL release.

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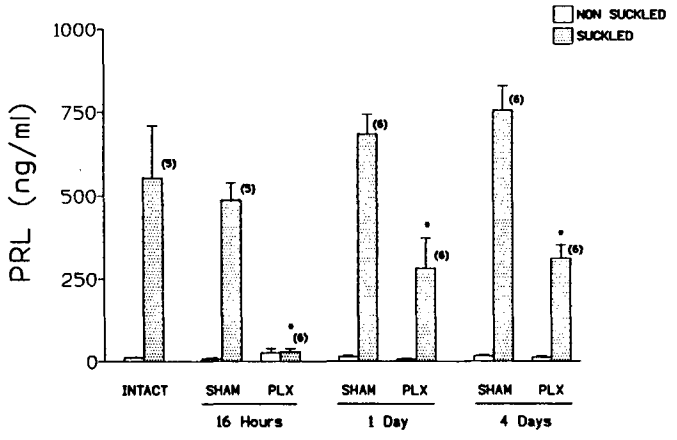

Fig. 1. Plasma PRL values (ng/mL) in intact, 16-h, and 4-d sham and 16-h, 1-d and 4-d lobectomized (PLX) rats, either nonsuckled for 8 h, or suckled for 15 min after 8 h nonsuckling. Numbers in brackets indicate number of rats in each group. Asterisks denote that p values were significantly less, i.e., $p < 0.05$ than values in sham and in intact rats.

Results

Effect of Suckling on Plasma PRL Concentration in Intact, Sham, and Posterior and Neurointermediate Lobectomized (PLX) Rats

Plasma PRL levels increased 25- to 30-fold above basal levels following 15 min of suckling in intact and in 16-h, 1-d, and 4-d sham rats, and about 10- to 15-fold above basal levels in 1- and 4-d PLX rats. However, no difference was observed between PRL levels of 16-h PLX nonsuckled and suckled rats, nor between basal levels of these rats and those of the other groups (Fig. 1).

The effectiveness of PLX to prevent milk ejection is shown in Table 1. Thus, in contrast to pups from intact and sham rats, or to pups from PLX rats injected with oxytocin (OXY) immediately before suckling, pups from PLX rats not injected with OXY did not obtain milk during suckling.

Effect of Suckling on Pituitary PRL Concentration

As shown in Fig. 2, a significant depletion transformation occurred in the central, but not in the peripheral region of the anterior pituitary of intact, 4-d sham and 4-d PLX suckled rats. No such effect was observed, however, in 16-h sham or in 16-h and 1-d PLX rats. In addition, it was found that AP PRL content of 16-h and 1-d PLX rats was significantly lower than those of the other groups.

In Vitro Secretion of PRL by Tissue Fragments from the Central and Peripheral Regions of the Pituitary Gland

Incubated AP fragments, both from the central and peripheral regions of the gland from 1-d PLX rats released approx 50% less PRL in 30 min than those of intact, 4-d sham and 4-d PLX rats (*see* Fig. 3). On the other hand, the central fragment of APs from intact and sham rats released 20–30% more PRL/mg AP than the corresponding peripheral region.

Table 1
Amount of Milk (g) Obtained by the Pups After 15 Min of Suckling[a]

Treatment	n	Milk, g
Intact	(6)	7.69 ± 0.85
16-h Sham	(5)	4.34 ± 0.43
4-d Sham	(6)	6.78 ± 0.70
16-hPLX	(6)	0.05 ± 0.03
1-d PLX	(6)	0.07 ± 0.05
4-d PLX	(6)	0.58 ± 0.20
	(5)	5.28 ± 1.33[b]

[a]The pups were first separated from the mothers for 8 h. Following this, the urine was expressed manually from the pups' bladders, and the litters were weighed to the nearest 0.5 g. Then to infer the completeness of posterior lobectomy by the lack of milk removal owing to endogenously released OXY, without injecting the hormone to the mother, the pups were allowed to suckle for 15 min and reweighed to estimate the amount of milk obtained.

[b]The mothers were injected with OXY (200 mU sc) immediately before suckling.

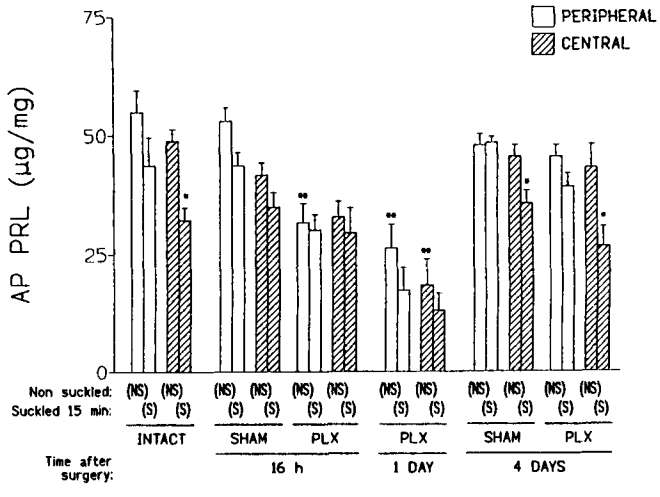

Fig. 2. Effect of suckling on PRL content (µg/mg) in AP fragments from the central, i.e., the inner region (empty bars) of the anterior pituitary surrounding the neurointermediate lobe, and the peripheral regions (hatched bars) of APs from the same rats as those in Fig. 1, i.e., intact, 16-h, and 4-d sham and 16-h, 1-d, and 4-d lobectomized (PLX) rats, either nonsuckled for 8 h (NS), or suckled for 15 min (S) after 8 h of nonsuckling. Single asterisk indicates $p < 0.05$ vs corresponding AP values from nonsuckled rats. Double asterisks denote that nonsuckled (NS) values were significantly lower, i.e., $p < 0.01$, than other NS values.

These regional differences were not observed, however, in AP fragments derived from 1- and 4-d PLX rats.

Discussion

The results of the present study indicate that a transient refractoriness to the PRL-releasing action of suckling occurs 16 h after the removal of the posterior and neuro-

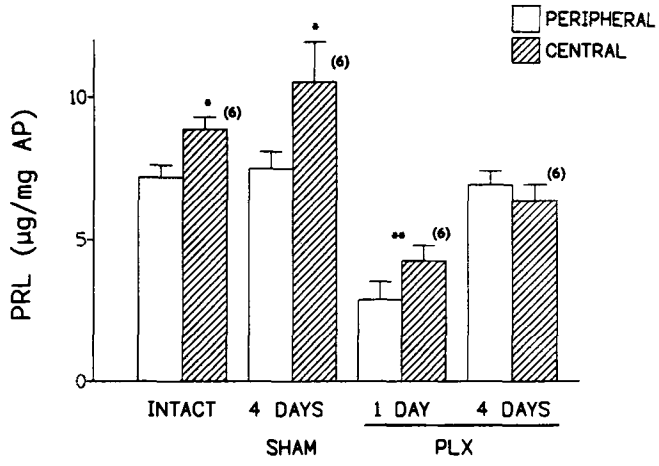

Fig. 3. Basal secretion of PRL (µg/mg) after 30 min of incubation from tissue fragments derived from the central or peripheral regions of APs from either intact and 4-d sham or 1-d and 4-d PLX rats, previously nonsuckled for 8 h. Single asterisks indicates $p < 0.05$ vs corresponding peripheral regions. Double asterisks denote that basal secretion by AP fragments from the 1-d PLX rats was significantly lower, i.e., $p < 0.05$, than all other values.

intermediate lobes of the pituitary gland of lactating rats. Following this period, there occurs a functional recovery during which suckling is again capable of stimulating the release of PRL, even though the effectiveness of the stimulus is decreased, as indicated by the significantly lower levels of the hormone in the circulation. In addition to these effects, it was found that, in agreement with previous results showing that the central AP region is more susceptible to PRL transformation, i.e., a decrease in PRL detectability (Grosvenor et al., 1979, 1980, 1984; Grosvenor and Mena, 1980; Mena et al., 1982, 1992, 1993; Lawson et al., 1987), than the peripheral region (Mena et al., 1993), suckling-induced PRL transformation occurred in the central AP region of intact, sham, and 4-d PLX rats, but not in APs of sham and 16-h and 1-d PLX rats. Since AP PRL content was significantly lower in 16- and 1-d PLX rats, this and the posttraumatic condition of the animals could account for the lack of changes in PRL content by suckling. Nevertheless, the fact that this phenomenon of transformation occurred in 4-d PLX rats suggests that its absence in the other groups was also part of a transient effect Therefore, as inferred from the results shown here, the posterior pituitary does not play a determinant role on PRL transformation, and although it exerts an effect on PRL release, such influence may be complementary to those from other sources (*see* Discussion section).

The acute and chronic effects of posterior and neurointermediate lobectomy on PRL release have been studied by different laboratories in rats under different conditions, i.e., lactating and cyclic (Fagin and Neill, 1982; Ben-Jonathan and Peters, 1982; Froehlich and Ben-Jonathan, 1984; Murai and Ben-Jonathan, 1986, 1987; Murai et al., 1989). In general, it was reported that in each of these conditions, increased basal levels of PRL, i.e., threefold to fourfold over presurgical levels, occurred immediately after PLX and then returned to normal either within a few hours after surgery (Ben-Jonathan and Peters, 1982; Froelich and Ben-Jonathan, 1984) or 3–4 d later (Fargin and Neill, 1982; Murai and Ben-Jonathan, 1986). In addition to these effects, it was reported that PLX of lactating rats was associated with ineffectiveness of suckling for inducing a phasic release of PRL (Murai and Ben-Jonathan, 1987). These findings were interpreted as owing mainly to removal of tuberohypophyseal DA inhibition and of a PRL secretagogue from the posterior and/or neurointermediate lobes, which would account, respectively, for the increased basal levels of the hormone after PLX and for the lack of effect of suckling on PRL release (Murai and Ben-Jonathan, 1987). Comparison of these results with those presented here shows that basal levels of PRL were not increased in our rats after PLX, even though higher, but not significant, levels of PRL were observed in the 16-h PLX rats. However, since in order to avoid additional stress our animals were not sampled frequently, and considering that after PLX increased, basal levels vary in magnitude and duration (Ben-Jonathan and Peters, 1982; Froehlich and Ben-Jonathan, 1984; Murai and Ben-Jonathan, 1986), we cannot exclude the possibility that such an effect may have also occurred to our rats before the experiment. Also, our finding that significantly lower levels of PRL content and in vitro release occurred in APs from 1-d and 16-h PLX rats, would indirectly support the possibility that postsurgical stress may have affected the functionality of the pituitary gland for PRL secretion. On the other hand, with respect to the inefficacy of suckling for PRL release in 16-h PLX rats in the present study, this result agrees with that quoted above of Murai and Ben-Jonathan (1987), in which PLX rats were suckled by the pups about 12–14 h after surgery. However, as also indicated above, this effect appears to be transient, since the effectiveness of suckling, although attenuated, was restored in the 1- and 4-d PLX rats. This suggests, therefore, that the anatomical integrity of the posterior pituitary is only in part essential for PRL release in the lactating rat, and that in the absence of this structure, PRL release is activated through PRFs from other sources. Thus, PRFs from the neurointermediate and posterior lobes (Murai and Ben-Jonathan, 1987; Hyde and Ben-Jonathan, 1988; Landon et al., 1990) may contribute, along with other factors, to regulating PRL release.

The possibility that the neurointermediate and/or the posterior lobes of the pituitary may influence regionalization of PRL release, i.e., increased release of PRL by AP fragments or cells from the central rather than from the peripheral regions of the AP (Boockfor and Frawley, 1987; Mera et al., 1993; Frawley, 1994), was analyzed by incubating AP fragments from 1- and 4-d PLX rats, and the results compared with similar tissues from intact and sham animals. The results obtained suggest that those structures

may indeed determine PRL regionalization, since contrary to what was observed in tissues from control rats, no differences in PRL release were observed in AP fragments from PLX rats.

Taken together, the results of the present and other studies suggest that the neurointermediate and/or the posterior lobes of the pituitary contribute by different mechanisms to the complex regulation of PRL secretion. Thus, according to the results of the present study, influences from the PNIL have no effect on suckling-induced PRL transformation, but they may have a partial effect on PRL release and may determine PRL regionalization. In this regard, drastic changes in responsiveness of lactotrophs to secretagogs have been reported to occur after suckling, mainly in those cells located within the central zone of the anterior pituitary (Hill et al., 1991; Porter and Frawley, 1992). Also, several studies have been made on the apparent release of α-MSH during suckling (Taleisnik and Orias, 1966; Hill et al., 1991; Nagy et al., 1991) and on the effect of this peptide and other PRFs on PRL release (Hyde and Ben-Jonathan, 1988; Landon et al., 1990; Hill et al., 1991, 1993; Mena et al., 1995). More work is required, however, to determine how these factors from the posterior and/or neurointermediate pituitary interact with influences from other sources, for the physiological release of PRL in lactating and nonlactating animals.

Materials and Methods

Animals

Primiparous lactating rats of the Wistar strain were used. They were kept in individual cages in a room with controlled light (lights on from 0700–2100 h) and temperature (23–25°C). Purina chow (Ralston Purina Co., Chicago, IL) and tap water were given ad libitum. Litter size was adjusted to 8–10 pups, and rats were used 7–10 d postpartum.

Surgery

Rats were anesthetized with ether, and the trachea was cannulated. Fifteen minutes before ether, to prevent excessive secretion in the respiratory tract, 0.03 mg sc atropine was administered to each rat. Removal of the posterior and neurointermediate lobes (PLX) of the pituitary gland or sham surgery (sham) was performed under a dissecting microscope, through the parapharyngeal approach; removal of the posterior and neurointermediate lobes by gentle aspiration via a bent needle, after undisturbed view, has been achieved. In essence, except for using ether as an anesthetic and making the hole for reaching the pituitary gland at the edge of the basosphenoid suture, the method employed was the same as that employed previously in other laboratories (Benson and Cowie, 1956; Fagin and Neill, 1982; Murai and Ben-Jonathan, 1987). Sham consisted of sectioning the dura and of insertion and withdrawal of the needle without application of suction. In general, the total time of anesthesia did not exceeded more than 15 min, and full recovery of the animals occurred within 30–60 min. Surgical procedures were performed either in the evening between 2200 and 2400 h (16-h groups) or in the morning between 0700 and 0900 h (1- and 4-d groups), at which time the mothers were reunited with their litters. The following experiments were performed.

Experiment 1

According to the time elapsed after surgery, the rats were divided into the following groups: intact, 16-h, 1-d, and 4-d sham-operated, and 16-h, 1-d, and 4-d posterior lobectomized (PLX). Between 0800 and 0900 h of the day of the experiment, the mothers were separated from their litters for 8 h, and then either sacrificed (nonsuckled control) or reunited with their pups for a 15-min suckling period. Milk yield was determined by weighing the pups before and after suckling, and then they were sacrificed. During the postoperative period, but not before the suckling test, milk was made available to the pups by injecting OXY (200 mU sc at 8-h intervals) to the PLX rats. Thus, the 16-h groups received a single dose of the hormone immediately after surgery, then the pups were separated for 8 h, and this was followed by the 15-min suckling test Also, by the same rationale, the 1-d groups received two doses and the 4-d groups received 11 doses of OXY prior to the 8-h separation period. The reason for not applying OXY before the suckling test was to exclude the possibility that this hormone by itself would induce PRL release. On the other hand, because the pups from PLX rats obtained very little milk after the suckling test, i.e., *see* Table 1, to exclude that low milk yields without OXY were not owing to depressed milk secretion, a group of 4-d PLX rats was injected with OXY before suckling. At the end of the experiments, rats were decapitated, trunk blood was collected and centrifuged, and the plasma was stored until assayed. Also, following decapitation, the hypothalamic–pituitary complex was inspected under a dissecting microscope. Only rats with a complete removal of the neurointermediate and posterior lobes and no damage to nearby structures, and for the suckled groups of rats, those in which the pups could not obtain any milk after the 15-min suckling, were included in the study. Following this, the AP of some animals was rapidly removed, dissected into the central, i.e., the inner region of the AP surrounding the intermediate lobe of the pituitary gland and the peripheral region of the gland (Boockfor and Frawley, 1987), weighed and frozen until assayed.

Experiment 2

To determine whether regionalization of PRL secretion, i.e., increased release of PRL by short-term incubated AP fragments from the central rather than from the peripheral region of the AP (Boockfor and Frawley, 1987; Mena et al., 1993; Frawley, 1994), was affected by posterior lobectomy, groups of intact, sham, and 1- and 4-d PLX rats were employed. These animals were treated the same as those in Experiment 1, and they were sacrificed at the indicated

times after surgery and following 8 h of nonsuckling. However, these animals were not suckled as those in Experiment 1 because as shown previously (Mena et al., 1993), suckling affects PRL regionalization. After each mother was sacrificed, APs were removed, dissected into the central and peripheral regions as indicated, and each region divided in half. One-half of each region of the AP from each rat was used to determine the initial concentration of PRL. The other half was rinsed, weighed, and further divided into two portions, which were incubated together in 0.3 mL of Earle's medium, pH 7.3 (Mena et al., 1982, 1992) in a metabolic shaker at 37°C, and under 95% O_2–5% CO_2 for 30 min. The medium was collected at the end of incubation, lyophilized, and along with tissue samples, stored frozen until assayed. The pre- and postincubated tissues were homogenized (with glass homogenizers) in 0.05*M* Tris-HCl buffer (pH 8.2). The AP fragments were immediately incubated on removal, since as previously shown (Mena et al., 1984), secretory dynamics of in vivo labeled PRL change rapidly as a function of time during incubation. Accordingly, incubated AP fragments were used rather than primary cultures of dispersed AP cells in order to prevent disruption of the hormone storage dynamics during the cell-disruption procedure.

PRL Determinations and Data Analysis

All plasma samples were analyzed for PRL by RIA within the same assay using materials kindly provided by the NIDDK (*see* Grosvenor et al., 1979; Mena et al., 1982 for details). The inter- and intra-assay coefficients of variation were 11.18 and 4.28%, respectively. Sensitivity of the RIA was 0.1 ng/ tube.

Tissue and medium samples from the in vitro experiments, as well as PRL concentration in APs from suckled and nonsuckled rats, were analyzed, as previously described (Mena et al., 1982, 1992), with the nondenaturing, nonreducing polyacrylamide gel electrophoresis (PAGE) densitometry procedures of Nicoll et al. (1969).

Owing to the fact that monomeric, i.e., 23-kDa, PRL appears as a single band in the lower gel after destaining, this method is most adequate to quantitate changes in AP content of this form of PRL, particularly those occurring during PRL transformation. Since this phenomenon of transformation occurs as a result of disulfide polymerization and phosphorylation of the PRL molecule at the expense of the monomeric form (Mena et al., 1982, 1992, 1993), a decrease in content of this form can be quantitated by PAGE densitometry, and thus it can be inferred whether PRL transformation has occurred or not. On the other hand, by contrast with this method, RIA measurements of PRL include not only the monomeric, but also other PRL variants having different degrees of immunoactivity (*see* Sinha, 1995 for review), and because of this, we considered RIA as being less adequate than PAGE for tissue analysis of PRL and limited its use only to plasma samples.

All data presented are means ± SEM. PRL concentration in plasma is expressed as ng/mL, whereas that in tissue and medium is expressed as μg/mg AP. Differences between means were compared by analysis of variance (one-way) followed by Tukey Kramer Multiple Comparisons Test. Statistical significance refer to a probability level of <5%, i.e., $p < 0.05$.

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