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AUTOCRINE REGULATION OF PROLACTIN SECRETION BY PROLACTIN VARIANTS RELEASED FROM LACTATING RAT ADENOHYPOPHYSIS

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Abstract

Background. Previous work has shown that the *in vitro* released prolactin (PRL) from the anterior pituitary (AP) of lactating rats, contains PRL variants i.e., from 7-14 to 70-97 kDa that when incubated with AP lactotrophs of male rats, and of rats in other conditions, they promoted the selective stimulation and/or inhibition of the *in vitro* release of PRL variants from APs of male rats and of rats in other conditions.

Methods. In the present experiments, we sought to determine whether PRL variants, released *in vitro* from lactotrophs of lactating rats, non-suckled (NS) for 6h or suckled (S) for 15 min after NS, were electroeluted from SDS-PAGE, and then were divided into 6 fractions, would influence the *in vitro* release of PRL variants from lactotrophs of NS and S rat APs.

Results. The results obtained showed that, under non-reducing conditions, the fractions contained PRL variants of 7-23 to 97 kDa, and between 1 and more than 20 ng/µl of PRL protein. Thus similar amounts of total PRL (about 60 ng/µl), were released from each AP region of NS and S rats, except for the higher amount of PRL (80 ng/µl) released from the central AP region of NS rats. The effects of PRL variants released from lactotrophs of NS and S rat APs i.e., of stimulatory and/or inhibitory type were exerted upon the release of PRL variants, and of total PRL.

Conclusions. These results indicate that in addition to hypothalamic influence, the release of PRL variants from the lactating rat AP is regulated also by autocrine influences exerted upon the gland by the previously released PRL variants.

Key words: Prolactin, anterior pituitary, lactation, lactotrophs.

INTRODUCTION

The synthesis and release of prolactin (PRL) by lactotrophs in the anterior pituitary (AP) are regulated by factors produced in the hypothalamus as well as in the posterior and neurointermediate pituitary lobes, by

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autocrine and paracrine signals from the anterior pituitary itself (for review see 1-5). In addition, it has been reported that total PRL and PRL variants are secreted under different physiological conditions (3-8) and it is known that functional interactions and cytological differences exist among pituitary lactotrophs within the anterior pituitary gland (2-7) and that functional variations (8-10), as well as autoregulation (11-12) and interactions with other pituitary cells (3, 7, 9) and with hypothalamic hormones (1, 3, 9) occur in different circumstances. For instance, lactotrophs from the central AP region (CR) of lactating rats, i.e., the region surrounding the neuro-intermediate pituitary lobe are bigger (10, 13), secrete more PRL than those of the peripheral AP region (PR) and after a short period of suckling become more sensitive to the PRL-stimulatory agents, TRH and angiotensin II. Moreover, they become unresponsive to dopamine; and interact with lactotrophs in the peripheral region of the gland (6, 13-15). In these studies, it is possible that the release of PRL variants may have influenced the regulation of PRL release.

In previous reports (13, 17) we showed that reconstituted conditioned media (RCM) and PRL variants i.e., from 7-14 and 70-97 kDa, from lactating rat APs, characterized by Western blotting and eluted from SDS-PAGE, promoted the *in vitro* vesicular release of the hormone from preformed, mature PRL granules of male rat APs, and that such release was independent of PRL synthesis (17). Autocrine and paracrine types of actions have also been shown to occur within the AP (7, 11, 12, 16, 17), and were demonstrated when the central and peripheral AP regions of lactating rats were incubated *in vitro* with RCM from pituitaries of lactating, pregnant and steroid-treated castrated males or females, but not from untreated castrated rats, intact male rats or by a PRL Standard (16, 17). Also, more potent effects occurred with RCM from APs of early- than from mid- or late- lactating rats and from rats non-suckled for 8 or 16 h than from those non-suckled for 32 h (17). These results suggest that, under certain conditions, PRL variants, released from lactating and non-lactating rat APs may regulate the release of PRL variants from the lactotrophs.

In the present study, RCM proteins, i.e., PRL variants, that were released *in vitro* from the AP regions of lactating rats, were separated and electroeluted from SDS-PAGE and tested using *in vitro* incubation techniques. We sought to determine first, whether PRL variants, which are known to occur within the AP (8, 18, 19) and are released *in vitro* (16, 17) after the suckling-induced PRL transformation, i.e., the transfer of the hormone from a pre-releasable to a releasable state (20, 21), would influence the release of PRL variants from lactating rat lactotrophs. Our results confirm that PRL variants are released into the CM from the central and peripheral AP regions of lactating rats, and that they interact and selectively and specifically stimulate or inhibit the *in vitro* release of other PRL variants from lactotrophs of lactating rat APs.

MATERIALS AND METHODS

General

Animal studies were performed under a protocol similar to the USPHS Guide for the Care and Use of Laboratory Animals and the Official Mexican Guide from Secretary of Agriculture (SAGARPA NOM-062-Z00-1999) published in 2001. Wistar primiparous lactating rats (8-10 pups per litter) were housed individually in a room with a reversed light-dark cycle (14 h light, 10 h darkness) and constant temperature (23-25°C) and were fed *ad libitum* (Purina Chow, Ralston Purina Co., Chicago IL, USA). On postpartum days 10-12 (7 am, local time) groups of mothers had their pups removed, and 6 h later their pups were or were not returned to the mothers and suckled for 15 min. At the end of the suckling or non-suckling periods, the mothers were killed by decapitation after light ether anesthesia. From all animals employed, the pituitary was removed and the posterior lobe was discarded using fine forceps as originally described by Bookfor and Frawley (10), the CR around the neurointermediate lobe and the PR i.e., the rest of the AP tissue (13, 15-17), were dissected independently, and incubated (see below).

Preparation of concentrated conditioned media

In individual flasks containing 300 µl of Earle's medium, media were conditioned by incubating tissue fragments corresponding to the CR and peripheral PR pituitary regions (13) from lactating rats. The pituitary fragments were incubated for 1 h, immediately after removal to prevent disruption of hormone storage dynamics (13, 20). Flasks containing the pituitary fragments were gassed with 95% O_2, 5% CO_2, sealed with rubber stoppers and incubated at 37°C in a water bath shaker (American Optical, Buffalo NY, USA). CM from pituitary fragments of each group of rats employed was concentrated and desalted in a Centricon micro-concentrator (Centripep, Millipore, Bredford MA, USA) and stored frozen until assayed, along with the corresponding tissue fragments.

Reconstituted conditioned media (RCM), and its incubation with pituitary fragments of lactating rats

Reconstituted conditioned media (RCM) was prepared by diluting 2 parts (2X) of concentrated CM in one part of Earle's medium. The PRL content initially present in the RCM was determined by ELISA, as described below. Subsequently, pituitary lactotrophs of lactating rats were incubated in 100 ml aliquots of either RCM or Earle's medium for 1 h. Finally, the total amount of PRL present in the medium, collected after the incubation period, was determined by ELISA. The PRL initially present in the RCM was subtracted from the total amount measured, giving the net amount of PRL secreted under each experimental condition.

Immunoprecipitation of PRL in RCM from lactating NS and S rat APs

Anti-PRL-IC-5 antiserum was obtained from the National Institute of Diabetes and Digestive and Kidney

diseases (NIDDK) and was used for immunoprecipitation. Information provided by the NIDDK, states that this antiserum possesses a high degree of species-specificity for RAT Prolactin, and that antibodies to other pituitary hormones are absent, as ascertained by demonstrating that binding of iodinated rPRL to this antiserum was inhibited by less than 0.5% in the presence of rGH, rFSH, rLH or rTSH. In our experiments, 200 µg of 2X RCM from AP regions of NS and S rats, and a control incubation containing 300 ng of PRL standard were each adjusted to 1 ml with dilution buffer (net-gel). The anti-PRL antibody was added to each sample and standard, and the tubes were incubated overnight at 4°C. The following day, 50 µL of Protein A-Sepharose was added to each tube. After incubating at 4°C for 3 h, the tubes were centrifuged for 5 min at 12,850 x g; the supernatants were saved and compared with the original RCM for their effects upon primary cultures of lactotrophs from lactating rats. The pellets were resuspended in 1 ml of net-gel buffer, incubated for 20 min at 4°C and centrifuged for 5 min at 12,850 x g. After discarding the supernatant, the pellets were resuspended in 1 ml of net-gel buffer containing 10% SDS followed by centrifugation for 5 min at 12,850 x g. The third treatment of the pellet consisted of adding 1 ml of TRIS-NP40 buffer (NONIDET P40, Bethesda Research Laboratories, Gaithersburg MD) incubating for 20 min at 4°C, and centrifuging for 5 min at 12850 x g. The washed pellets were resuspended in 20 µL of Laemmli's buffer, heated at 100°C for 5 min in water bath shaker, and then centrifuged to remove the Sepharose. Following this the PRL content in the samples was determined by ELISA.

Deglycosylation of PRL

The native protein Deglycosylation Kit (Cat. NDEGLY, Sigma, St. Louis, MO USA) was employed. To 200 µg of dehydrated conditioned media of the central region AP from non-suckled and suckled lactating rats, 37.5 µL of deionized water was added, followed by 10 µL of 5X reaction buffer (Cat. No. R9025 Sigma) and 2 µl of Endoglycosidase F1 from the Kit (ENDO). In parallel, another tube containing conditioned media was prepared in the same way, but without endoglycosidase. Both tubes were then incubated for 1 h at 37°C, and then tested for their effect upon PRL release on primary culture lactotrophs from male rat APs, and then determined by ELISA.

Dephosphorylation of PRL

An aliquot containing 200µg RCM protein of the central AP region from non-suckled and suckled lactating rats of the central region AP were incubated with 0.1 U of acid phosphatase (FAH), human prostate affinity purified (Sigma), dissolved in 20 µL of 0.1M sodium acetate buffer (pH 5), and 20 µLof acetate buffer was subsequently added. After incubating at 37°C for 2.5 h, 100 µl of PBS was added to each tube, and the samples were washed in a Centricon-10 tube, first with PBS and then with deionized water, and finally they were vacuum dried. Two parallel samples, one without FAH and another with FAH were treated in the same way. Then tested for their effect upon PRL release on primary

culture lactotrophs from male rats, and then determined by ELISA.

SDS-PAGE

In the present and previous studies (15-17) samples were analyzed by non-denaturing (NR) conditions SDS-PAGE, 12.5% gels using the buffer system and Bradford (22) in a mini Protean III cell (Bio-Rad). Samples were electrophoresed under NR conditions. The gels were divided into 6 fractions which as shown previously (17) encompass PRL variants from 6 to 97 kDa. The proteins in each fraction were electrophoretically eluted, dialyzed, lyophilized, and then assayed by ELISA for PRL content as well as for their effects upon PRL secretion on primary culture of pituitary cells from lactating rats.

Enzyme-linked immunoabsorbant assay (ELISA)

The concentration of PRL in all conditioned media and in the eluates from the SDS-PAGE gels was determined by the enzyme-linked immunoabsorbant assay (ELISA) method as modified by Signorella & Hymer (23). Briefly, 96-well microtitre plates (Immulon 2HB, Chantilly VA, USA) were coated overnight at 4°C with 10 ng of rat PRL in 100 ml of 1 M carbonate buffer, pH 10.3. The plates were washed with TPBS (0.01 M sodium phosphate, 0.15 mM NaCl, 0.05% v/v Tween-20, pH 7). This washing procedure was performed after each incubation step. For the standard curve, serial dilutions of rat PRL (NHPP-NIH) (0.06 to 64 ng/ml) in TPBS, were incubated for 16h with 100 ml primary anti-rPRL polyclonal antiserum (1:40000; NHPP-NIH) in TPBS containing 1% (w/v) non-fat dried milk (Bio-Rad). Samples and standards (100 ml) were then added to the coated wells and incubated for 2h at room temperature. Secondary goat anti-rabbit IgG peroxidase conjugate (Bio-Rad) was then added (1:3000 in TPBS with 1% non-fat dry milk) and incubated for 2h at room temperature. Bound secondary antibodies were detected by reaction with 2, 2'-amino-di-[3-ehylbenzothiazoline sulfonate] substrate (Roche, Mannheim, Germany). Plates were read 15 min later in an automatic ELISA Microplate Reader (Bio-Rad) at 405 nm. The assay has a sensitivity of 2 ng/well and inter-assay and intra-assay coefficients of variation of <6%.

Primary cultures of pituitary cells

Lactating rat pituitary fragments (n=5) corresponding to the CR and PR of the anterior pituitary (10, 13) were dissected and processed separately. Primary cultures were prepared as described by Fiordelisio & Hernandez-Cruz (24). Briefly, the central and peripheral regions of the anterior pituitary of NS and S rats were separated, cut into pieces, and digested by incubation with 2.5 mg/ml trypsin and 0.1 mg/ml collagenase (Worthington Biochem Co; Lakewood NJ, USA) in DMEM. The 6 tissue fragments were then gently triturated with a Pasteur pipette. The cells were collected by centrifuging for 10 min at 185 x g, and washed twice with DMEM containing 10% BSA. The pellet was resuspended in DMEM, supplemented with 10% horse serum, 2% Fetal Bovine

Serum, 10,000 U penicillin, 10 mg/ml streptomycin, all from Gibco BRL, Grand Island NY, USA. The cultures were maintained for 24 h at 37°C in a humidified atmosphere (95% air and 5% CO_2). The primary cultures were placed in the bottom of 24-multiwell culture plates (Costar, Cambridge, MA, USA) at a density of 2 x10^4 cells per well and three replicates were used in the experimental system.

Statistical analysis

The PRL concentration was calculated by linear regression and values of PRL concentration obtained by ELISA were averaged for each experimental group. Statistical differences were determined by a one-way analysis of variance (ANOVA), using Dunnett's test, and compared all treatments versus control (Earle's medium). Comparisons were analysed with the Graph Pad Prism, version 5.0 Software, Inc. (San Diego, CA). The significance level was set at $p<0.05$. Each control or test compound was assayed in duplicate, and the assays were performed three times (n=3).

RESULTS

Immunoprecipitation of PRL contained in RCM from lactating rats prevents its effects upon lactating rat pituitaries

As shown in Figure 1 (A-B), 2X RCM from both AP regions, i.e., peripheral (PR) and central (CR) of non-suckled (panel A) and suckled (panel B) rats, provoked a clear stimulatory effect on PRL release from lactotrophs of both regions, and these effects of RCM were prevented by immunoprecipitation with anti-rat PRL.

Effect of deglycosylation and dephosphorylation of RCM proteins from NS and S lactating rats upon PRL release from lactating rat lactotrophs.

The effect of endoglycosidase (ENDO) and of acid phosphatase (FAH) on each RCM of NS (panel A) and S (panel B) rats, i.e., Figs. 2 and 3 (A-B), upon the effect of PRL release from lactotrophs from APs of lactating rats resulted in a significant increase in the released PRL.

PRL content of electroeluted PRL variants released from AP regions of lactating non-suckled and suckled rats.

As shown previously (15, 17) the PRL variants (fractions) released from each AP region of NS and S rats were analyzed previously by SDS-PAGE and Western blotting. In the present study the fractions 1-6 from SDS-PAGE were EE, its EE PRL content was determined by ELISA as shown in Fig. 4 A-D, and subsequently, to determine whether their presence had an effect i.e., increased and/or decreased released of PRL variants, they were incubated with lactotrophs of each AP region of NS and S rat APs i.e., Figs. 5-8 (A-B). The fractions contained bands of 7-23 to 97 kDa under NR conditions, and between 1 and more than 20 ng/μL of PRL protein. However, in spite of these variations, similar amounts of total PRL (about 60 ng/μL right panels), were released from each AP regions of NS and S rat APs, except for the higher amount (80 ng/μL) released from the

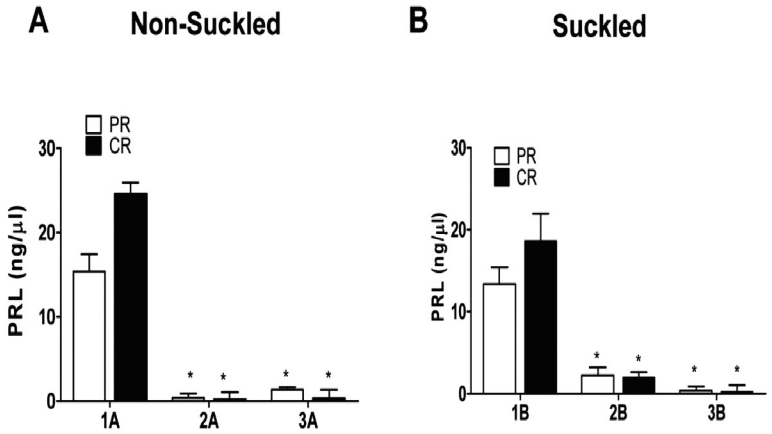

Figure 1 (A-B). Samples for 1 A and 1B of Reconstituted Conditioned Media (RCM) from non-suckled (panel A) and suckled (panel B) rats, without antibody. 2A and 2B, RCM were the supernatants of RCM's preincubated with antibody against PRL as described in the Methods Section; 3A and 3B were controls corresponding to Earle's medium. All samples were tested for their ability to stimulate PRL release upon lactotrophs from male rat APs. Data are means ± SEM. *Differences $P < 0.05$ *versus* no treatment with antibody, (n=3).

central AP region of NS rats .

Autocrine effects of electroeluted PRL variants, released from AP regions of lactating non-suckled rats, upon the in vitro release of PRL variants from lactotrophs of NS rats.

The EE PRL variants (fractions) that were released from each AP region of NS rats (Figs. 4 AB) were tested for their effects upon the amount of PRL released from AP regions of NS rats, and the results are shown in Fig. 5 A-B. The PRL content in fractions from the PR region of NS rat APs (Fig. 5A) was low <3 ng/µL of PRL in fraction 2, and high (about 10 ng/µl) in fractions 1 and 3-6; and with respect to the total PRL, upon incubation with the fractions were released from the lactotrophs of the same, i.e., the peripheral AP region of NS rats, increased release i.e., 5-7 ng/µL, occurred to PRL variants 1, 2, 4 and 6, and decreased release to 3 and 5. When incubated with EE fractions from the PR, lactotrophs from this same region showed increased release of PRL in fractions 1, 2, 4 and 6, but lower release in 3 and 5. However, except for the amounts of PRL released from fractions 2 and 6, whose levels were higher or similar than those of the other fractions, the amounts of PRL released by the other variants were significantly lower than those of the EE PRL. When the central region of NS rats was incubated with the fractions from the peripheral AP regions of NS rats,

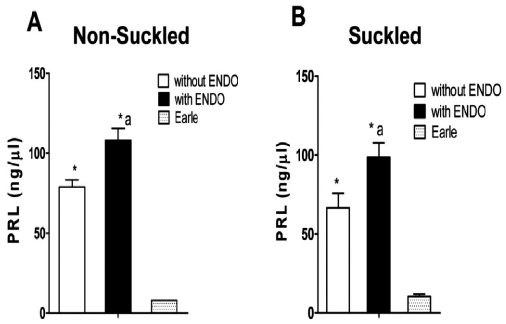

Figure 2 (A-B) . Samples of RCM from the central AP region of non-suckled (panel A) and suckled (panel B) lactating rats were incubated with and without endoglycosidase (ENDO) as described in the Methods Section, and were tested for the ability to stimulate PRL release from lactotrophs of primary cultures of male rat APs. Data are means ± SEM. *Differences $P< 0.05$ *versus* control (Earle's medium); Letter a indicates $P<0.05$ *versus* no enzymatic treatment.

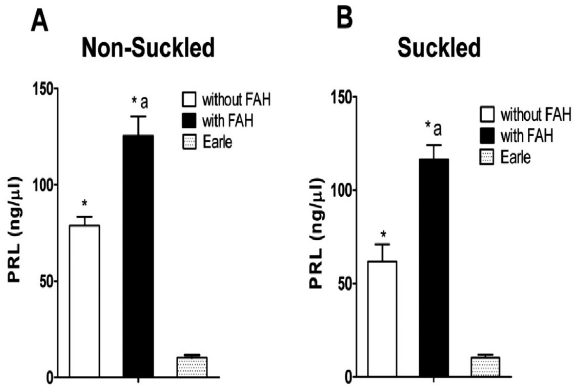

Figure 3 (A-B) . Samples of RCM from central AP region of non-suckled (panel A) and suckled (panel B) lactating rats were incubated respectively, with and without acid phosphatase (FAH) as described in the Methods Section, and were tested for their ability to stimulate PRL release from lactotrophs of primary cultures of male rat APs. Data are means ± SEM. *Differences $P< 0.05$ *versus* control (Earle's medium); Letter a, indicates $P<0.05$ *versus* no enzymatic treatment.

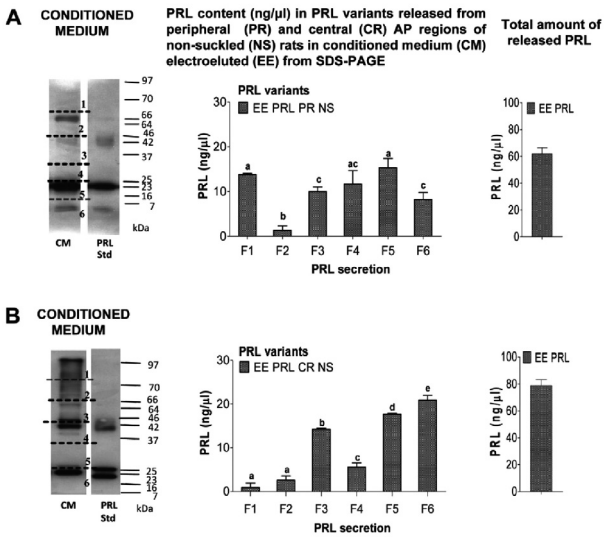

Figure 4 (A-B). SDS-PAGE (left panels) and prolactin (PRL) content (ng/µL) of PRL variants (middle and right panels) released from the peripheral (PR) and central (CR) adenohypophyseal (AP) regions of non-suckled (NS) rats, and electroeluted (EE PRL) from fractions 1-6 of SDS-PAGE. Data are means ± SEM. Letters (a-d) indicates $P < 0.05$ difference between fractions of EE PRL.

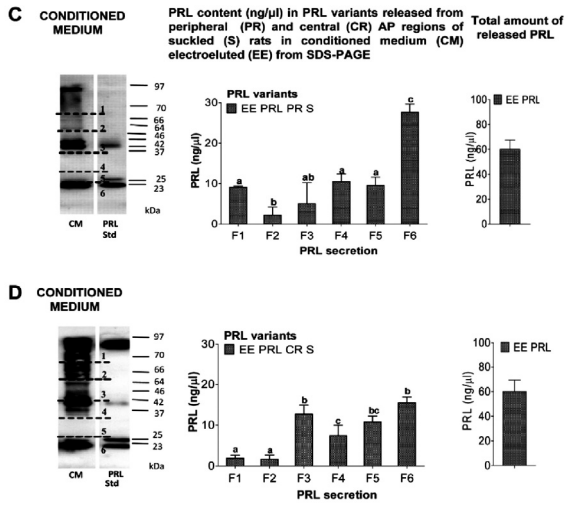

Figure 4 (C-D). SDS-PAGE (left panels) and prolactin (PRL) content (ng/µL) of PRL variants (middle and right panels) released from peripheral (PR) and central (CR) AP regions of suckled (S) rats and electroeluted from fractions 1-6 of SDS-PAGE. Data are means ± SEM. Letters (a-d) indicates $P < 0.05$ for the difference of PRL content between electroeluted fractions.

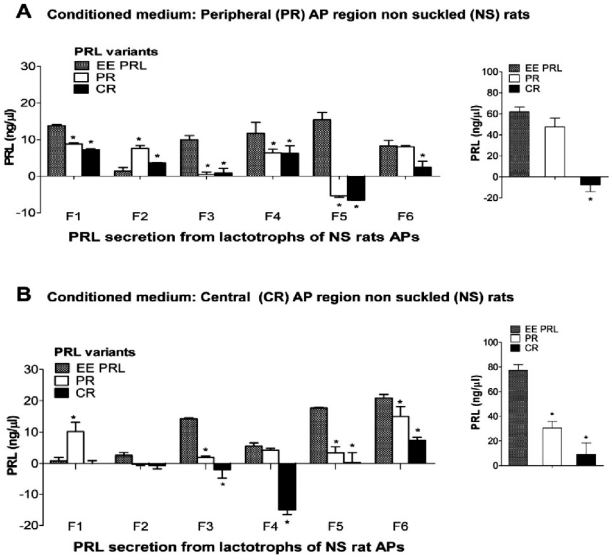

Figure 5 (A-B). Effect of the PRL variants electroeluted (EE PRL) in fractions 1-6 from SDS-PAGE of RCM from the peripheral (PR) **(panel A)**, and central (CR) **(panel B)**, AP regions of non-suckled (NS) lactating rats upon the *in vitro* release of PRL variants by lactotrophs from AP regions of NS lactating rats. Data are means ± SEM. *Differences $P < 0.05$ *versus* control (EE PRL).

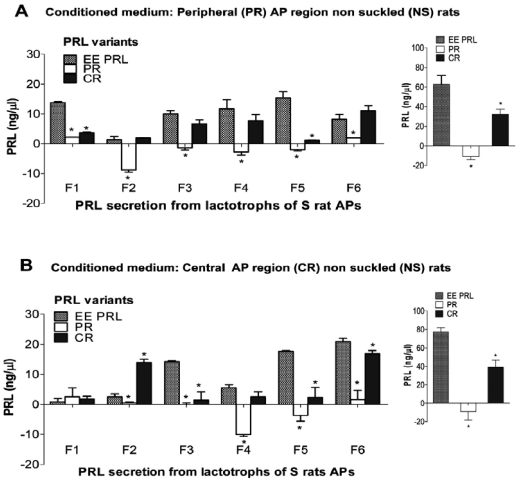

Figure 6 (A-B) . Effect of the PRL variants electroeluted (EE PRL) in fractions 1-6 from SDS-PAGE of RCM from the peripheral (PR) **(panel A),** and central (CR) **(panel B)**, AP regions of non-suckled (NS) lactating rats upon the *in vitro* release of PRL variants by lactotrophs from AP regions of suckled (S) lactating rats. Data are means ± SEM. *Differences $P < 0.05$ *versus* control (EE PRL).

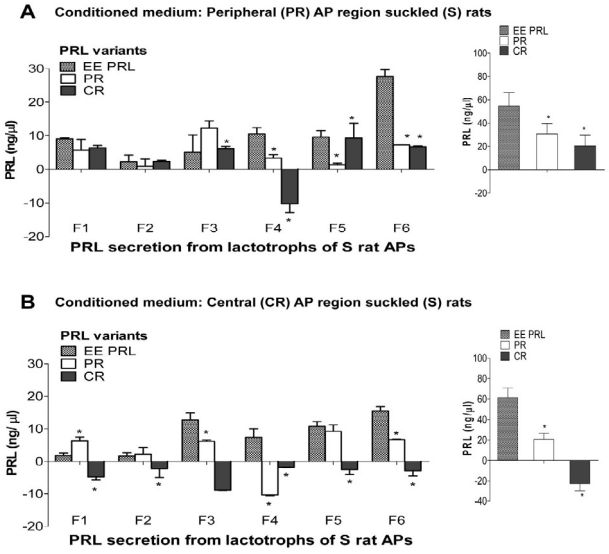

Figure 7 (A-B) . Effect of the PRL variants electroeluted (EE PRL) in fractions 1-6 from SDS-PAGE of RCM from the peripheral (PR) **(panel A)**, and central (CR) **(panel B)**, AP regions of suckled (S) lactating rats upon the *in vitro* release of PRL variants by lactotrophs from AP regions of suckled (S) lactating rats. Data are means ± SEM. *Differences $P < 0.05$ *versus* control (EE PRL).

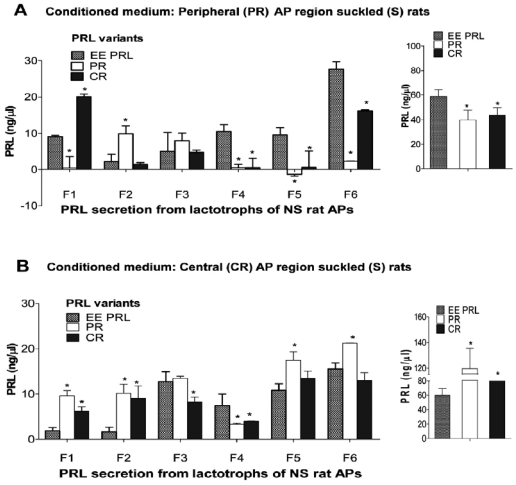

Figure 8 (A-B) . Effect of the PRL variants electroeluted (EE PRL) in fractions 1-6 from SDS-PAGE of RCM from the peripheral (PR) **(panel A)**, and central (CR) **(panel B)**, AP regions of suckled (S) lactating rats upon the *in vitro* release of PRL variants by lactotrophs from AP regions of non-suckled (NS) lactating rats. Data are means ± SEM. *Differences $P < 0.05$ *versus* control (EE PRL).

medium levels (about 8 ng/μL PRL), occurred to fractions 1 and 4, and medium to low levels to 2, 3, 5, and 6. As a result of these effects high levels of total PRL, not different than those of the EE PRL variants, were released from the peripheral but not from the CR, from which the release of total PRL was significantly depressed, below the initial level.

In figure 5B, the PRL content of the EE fractions released from the central AP region of NS rats was low in fractions 1, 2 and 4 of CM, and high in 3, 5 and 6, i.e., above 10 ng/μl. Also, with respect to the effect of incubating lactotrophs from the central AP region of NS rats with the EE PRL variants from the peripheral AP region, increased release occurred only of PRL variants 1 and 6; low levels occurred to 1-5 from the central AP region; and only the fraction 6 was above the zero level, i.e., about 5 ng/μL of PRL. Overall, significantly lower levels of PRL than those contained both in the EE PRL variants and in those released from the peripheral region, were released from lactotrophs of both the central and peripheral AP regions.

Autocrine effects of electroeluted PRL variants released from AP regions of lactating non-suckled rats upon the in vitro release of PRL variants from lactotrophs of suckled rat APs.

The effect of incubating lactotrophs from AP regions of S rats with EE fractions (PRL variants) released from AP regions of NS rats is shown in figures 6 A-B. In figure 6A, i.e., upper panel, the PRL content in fractions from the PR of NS rats (c.f. Fig 4 A-B) was low in fraction 2, and showed medium to high levels, (about 10 ng/μl) in fractions 1, 3-6; the amount of PRL released from the same AP region of S rats was around zero in fractions 2-5; and low 1 and 6. As a result, significantly lower amounts of PRL were released from the PR of S rats. Also, as shown in figure 6B, the amount of PRL released from the CR of NS rat APs showed low levels in fractions 1, 2 and 4 and high levels in 3, 5 and 6; the total amount of released PRL from the PR was zero in all fractions, and thus, it was significantly lower than that of the EE PRL. With respect to the amount of PRL released from the CR, only fractions 2 and 6 showed high levels and the levels of the other fractions were much lowers (1-3 ng/μL). As a result of this, the amount of released PRL from the PR of S rats, was lower than zero, i.e., lower than that of the EE control PRL, and of the still lower amount of PRL released from the CR.

Autocrine effects of electroeluted PRL variants released from AP regions of lactating suckled rats upon the in vitro release of PRL variants from lactotrophs of suckled rat APs

The effect of incubating lactotrophs from AP regions of S rats, with fractions i.e., EE PRL variants, released from AP regions of S rats is shown in figures 7 A-B. As shown in figure 7A, the PRL content from the PR (c.f. Fig. 4C-D) showed medium to high levels, i.e., 5 to more than 20 ng/μL, in fractions 1, 3, 4-6, and a lower level in 2; and the amount of PRL released from the peripheral AP region of S rats was medium to high in fractions 1, 3 and 6 and medium to low in 2, 4 and 5; and

with respect to the amount of PRL released from the CR of S rats (figure 7B), low and medium levels (2 and 8-10 ng/μL), occurred in fractions 1, 2 and 3-6, respectively; and with respect to the effect of incubating lactotrophs from the CR of S rats with EE PRL variants from the same AP region of S rats, the levels were low in fractions 1 and 2 and higher (around 10 ng/μL PRL) in 3-6; and medium to low levels (1-6 ng/μL) occurred in fractions 1 and 2, reduced levels (-10 ng) in 4 and higher levels (8-10 ng/μl) in 3, 5 and 6; with respect from the peripheral AP region of S rats, below zero levels, were released from fraction 4 of the same AP region; and there was only a small stimulatory effect on fractions 1, 2, 3, 5, and 6. EE PRL reduced the release of PRL from the CR to below zero levels in all fractions. As a result of these effects, the total amount of PRL released from the PR, and particularly from the central AP region, was significantly lower than that of both the EE PRL variants and of the amount released from the peripheral AP region.

Autocrine effects of electroeluted PRL variants, released from AP regions of lactating, suckled rats, upon the in vitro release of PRL variants from lactotrophs of non-suckled rat APs

The effect of fractions (PRL variants) released from AP regions of suckled rats upon the release of PRL from lactotrophs of the PR and the CR of NS rats is shown in figures 8 A-B. In Fig. 8A, the PRL content of the EE fractions from the RCM of the peripheral AP regions of S rats, i.e., Fig. 4C-D, was low to medium in fraction 2 and 3, medium to high in 1, 4 and 5, and particularly high in fraction 6; and as a result of incubation, the amount of PRL released from lactotrophs of the PR of NS rats was low in fractions 1, 4-6, and high only in 2 and 3; and from the CR of these rats, the amount of PRL released was particularly high in fractions 1 and 6; medium in 2 and 3, and low in 1 and 4-5. As a result of these interactions, the amount of total PRL released from both AP regions was significantly lower than the amount EE PRL, but higher than that shown from NS and S rat AP regions, (c.f. Figs. 5-7 A-B), due to the effect of RCM from NS and S rats.

In figure 8B, the PRL content of the EE fractions from the central AP region of S rats, was low in fractions 1 and 2, and high in 3-6; however, the amount of PRL released mainly from the peripheral, and in part also by the CR, was higher by the peripheral than the EE PRL in fractions 1, 2 and 5, 6; this level was about the same in 3, and lower in 4; and with respect to the amount of PRL released from the central AP region it was higher than the EE PRL in fractions 1 and 2, lower in 3 and 4, and about the same high level in fractions 5 and 6. As a result of these effects, an increased release of the hormone PRL occurred from both AP regions, particularly from the PR, whose levels, except from that of fraction 3, were significantly higher than those of the EE PRL as well as of that released from the CR, whose levels in fractions 1 and 2 were also higher than those of the EE PRL.

DISCUSSION

This study confirms that PRL variants ranging from 7 to 97 kDa, are released from the lactotrophs of each AP region of the anterior pituitary gland of NS and S lactating rats, as determined by SDS-PAGE (present study) and by both SDS-PAGE and Western blot (15, 17). When these variants are electroeluted from SDS-PAGE and then incubated with lactotrophs from each AP region of the same type of rats, they exert different effects (promotion, inhibition or no effect) upon the release of PRL variants from lactotrophs of both AP regions of NS and S lactating rats. Thus, these results indicate that autocrine regulatory effects are exerted by different fractions (PRL variants) upon the release of other variants of the hormone from lactating rat APs, and they are in accord with previous studies showing autoregulation of PRL secretion (11, 12, 16, 17). Similar effects on male lactotrophs with RCM from pregnant and lactating females and steroid-treated castrated males or females, but not from intact males or by a PRL standard were reported previously (16, 17).

In the present study, the effects upon the release of PRL from lactating rat lactotrophs were caused by the fractions (PRL variants) electoeluted from SDS-PAGE, and the PRL content was quantified by the ELISA method. Also, in support of this was the fact that the immunoprecipitation of PRL contained in RCM from lactating rats, prevented the effects of PRL variants upon PRL release. The PRL contained in the fractions contained PRL variants, ranging from 7 to 97 kDa, as revealed by Western blotting under NR conditions and from 7 to 66 kDa under R conditions, as shown previously (16, 17). Prior to fractionation the total PRL variants from both, the central and peripheral AP regions of NS rats stimulated the release of PRL from the peripheral, but inhibited its release from the central AP region. However, when RCM from lactating rats was fractionated by SDS-PAGE, eluates from the fraction 6 containing 23-25 kDa PRL had the greatest effect on PRL release, although weaker immunoreactive bands with lower, or even inhibitory activity, were also detected in the upper gel fractions. In addition, RCM from the lactating rat pituitary contains several variants i.e., 37 to 46 kDa as well as 23 to 25 kDa PRL variants, that exert different effects upon the release of other PRL variants from the lactating rat pituitary (present study) and from APs of rats in different conditions (15-17).

Therefore, the present study shows that the lactating rat pituitary produces PRL variants that are absent or deficient in the male pituitary gland and in the PRL Standard, even though the AP of male and of other types of rats, do respond to stimulatory factors released from the anterior pituitary of lactating rats (17). The results presented here, together with those in our previous study, also indicate that several PRL variants are produced and released by the lactating rat pituitary (2, 8, 15); this hormonal heterogeneity may be physiologically very relevant in the context of autoregulatory mechanisms determining the wide range of PRL effects under different physiological conditions (8, 15-17) and upon different structures (1, 3, 4).

In addition to the regulatory effects

of PRL variants from lactating rats upon the release of the hormone, further evidence of these effects was obtained in the present study, when RCM's from lactating rats were treated with phosphatase or with endoglycosidase which increased their ability, i.e., that of the PRL variants to stimulate PRL release from lactating rat APs, similar to the effect shown previously upon male rat lactotrophs (17). These effects of dephosphorylation and deglycosylation of RCM provide additional evidence that PRL variants in RCM are responsible for the effects upon lactating rat lactotrophs. As shown previously, PRL released from the AP of lactating and non-lactating rats (15, 17) is phosphorylated and glycosylated and thus, it is less bioactive than the dephosphorylated and deglycosylated variants (19, 21, 25, 26).

In conclusion, the results of the present and previous studies suggest that, the regulation of the release of PRL variants from the lactating rat AP is regulated also by autocrine influences exerted upon the gland by the previously released PRL variants.

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