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Depletion and Release of Prolactin from Rat Pituitaries and Pituitary Tumors *in Vitro*[1] (42809)

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Abstract. Depletion of pituitary prolactin (PRL) and PRL release into culture medium were simultaneously examined over a 3.5- to 4.0-hr incubation period from anterior pituitary fragments obtained from Fischer-344 or Wistar–Furth female rats treated with estrogen for 5 days, in pituitary tumors induced by 8 weeks of diethylstilbestrol (DES) treatment in Fischer-344 rats and in MtTW15 pituitary tumors transplanted subcutaneously in Wistar–Furth rats for 4 weeks. Our objective was to determine if the event known as transformation, which we define as a loss in the tissue PRL content without a corresponding and equivalent increase in the medium PRL content, occurs in rat pituitary tumors. Our results indicated that transformation did not occur *in vitro* in rat anterior pituitary tumors induced in Fischer-344 rats by DES treatment but was present in pituitaries from Fischer-344 rats treated for 5 days with estrogen, which served as controls. We also observed *in vitro* transformation in the anterior pituitary of Wistar–Furth rats treated with estrogen for 5 days (controls) and in the pituitaries of Wistar–Furth rats inoculated with the MtTW15 tumor for 4 weeks, but not in the MtTW15 tumor itself. Although transformation was present in both Fischer-344 and Wistar–Furth rats treated acutely with estrogen the timing of the transformation was delayed 1–2 hr in the Fischer-344 rats compared with Wistar–Furth females. We concluded that transformation does not precede release of prolactin in rat pituitary tumors and that in normal pituitaries the mechanisms of transformation are induced differently between the strains of rats examined.

Nicoll and his colleagues (1, 2) proposed that pituitary prolactin (PRL) is converted (or transformed) from a storage pool to a releasable pool prior to release. This mechanism is manifested *in vitro* as a loss in the tissue PRL content without a corresponding increase in the level of PRL in the medium. Mena and his co-workers (3) verified and extended Nicoll's *in vitro* findings by showing that pituitary PRL of nonsuckled lactating rats depletes and repletes rapidly in a cyclic fashion *in vitro* whereas release is steady. This same group related depletion to the age of PRL (4) and to a change in extractability which was pH dependent (3) or related to intramolecular thiol–disulfide exchanges (5).

Swearingen (6) also provided early evidence for an *in vitro* transformation-like response in the anterior pituitaries obtained from estrogen-treated rats which she concluded was the result of transfer of PRL between at least two functional pools within the lactotroph. In addition, we have presented *in vivo* evidence for PRL transformation in ovariectomized rats supplemented with 2 days of estradiol benzoate (7).

The objective of the *in vitro* study reported here was to compare pituitary PRL depletion and release from anterior pituitary tumors with that of normal pituitaries from Fischer-344 and Wistar–Furth rats that were ovariectomized and treated with estrogen. The Fischer-344 rats were chosen because these rats are extremely sensitive to estrogens and they develop hyperplastic pituitaries within 8–10 weeks of chronic estrogen treatment (8). The Wistar–Furth strain was selected as a strain that can carry the transplantable MtTW15 pituitary tumor.

Materials and Methods. *Animals.* Sexually mature (150–200 g) female Fischer-344 (Charles River, Portage, MI) and Wistar–Furth (Harlan–Sprague–Dawley, Inc., Indianapolis, IN) rats were used in these studies. Animals were housed two per cage under controlled lighting (artificial illumination 14 hr light, 10 hr dark; lights on at 0600 hr),

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temperature (23°C), and humidity (50%). Animals had access to tap water and standard rat chow (Purina, St. Louis, MO) *ad libitum*. All except one group of Wistar–Furth rats were ovariectomized (OVX) 2–7 days after their arrival in the laboratory. Groups of ovariectomized rats were then injected subcutaneously with 100 μg polyestradiol phosphate (PEP, Estradurin, Ayerst Laboratories, New York, NY) on the day of OVX or implanted subcutaneously on the day of OVX with a 5.0-mg pellet of diethylstilbestrol (DES, Innovative Research of America, Rockville, MD) (Fischer-344). The latter group was sacrificed 8 weeks after DES implantation.

The group of Wistar–Furth rats that was left intact was inoculated (sc) with a 1.0-ml suspension of the minced tumor obtained from a donor MtTW15 tumor-bearing animal. The recipient tumor-bearing rats were sacrified 4 weeks later.

Experimental protocol. In all experiments the rats were sacrificed by decapitation following a 1-min period of ether anesthesia and the anterior pituitary or tumor was rapidly removed, sectioned into four 2- to 5-mg fragments which were placed on a moistened paper towel, and transferred from the animal quarters to the tissue culture laboratory. One pituitary fragment was placed in each well of 24-well tissue culture plates (Corning Glass Works, Corning, NY) which contained 1.0 ml of minimal essential medium (MEM, Sigma Chemical Company, St. Louis, MO) buffered with sodium bicarbonate (26 m*M*). Dopamine was not included in the medium. The plates were incubated at 37°C in a humified environment of 95% O_2–5% CO_2 for 3.5 or 4 hr. At 30-min intervals 8–10 fragments were removed from representative wells and were snap frozen on dry ice. The fragments snap frozen at the beginning of the incubation (0 min) were placed in medium for 30 sec prior to freezing the tissue. The remaining fragments were transferred into wells containing fresh medium. In one experiment representative anterior pituitary fragments were also removed and frozen at 10 min of incubation and in another tissue was removed at 30, 120, and 240 min of incubation.

At the end of the incubation period, all medium samples were frozen at −20°C until the time of the PRL assay. The frozen tissue fragments were quickly thawed, weighed, and then sonicated for 20 sec in either 1.0 ml phosphate-buffered saline (PBS, pH 7.6) or 1.0 ml 0.05 *M* $Na_2CO_3/NaHCO_3$ (pH 10.0). The sonicates were placed at 4°C for 1 hr and then each was diluted 1:100 with PBS/0.1% bovine serum albumin (BSA, Pentax, Miles Laboratories, Naperville, IL) and stored at −20°C until the time of the PRL assay.

PRL assay. All medium samples and tissue sonicates were assayed for PRL at two dilutions in duplicate by double antibody radioimmunoassay (RIA (9)). NIDDK-RP-I5, iodinated using the lactoperoxidase–glucose oxidase method of Tower *et al.* (10), was used as a tracer. The standard was NIDDK-RP-1 (11.0 IU/mg).

Statistical analysis. Least-squares polynomial regression analysis was done to determine the rate of loss of pituitary PRL content and the rate of release into the medium. The coefficient of determination for each regression was tested for significance by an *F* test.

Results. Time-dependent *in vitro* depletion and cumulative release of PRL from pituitaries of ovariectomized Fischer-344 rats treated for 5 days with polyestradiol phosphate are shown in Fig. 1. Depletion of pituitary PRL best fit a fourth-order polynomial regression with the most marked depletion (approximately 8 μg/mg AP) occurring between 90 and 210 min of incubation. Cumulative release was best approximated by a second-order polynomial regression. Approximately 8 μg/mg AP accumulated in the medium over the $3\frac{1}{2}$-hr period; however, 1.3 μg/mg AP accumulated during the period when pituitary PRL was depleted by 8 μg/mg AP (90–210 min).

When pituitaries from Fischer-344 rats treated for 8 weeks with diethylstibestrol were incubated as above (Fig. 2), pituitary PRL was not significantly depleted over the $3\frac{1}{2}$-hr period but approximately 1.5 μg/mg AP of PRL accumulated in the medium. Release best fit a second-order polynomial regression.

In the third experiment pituitaries from

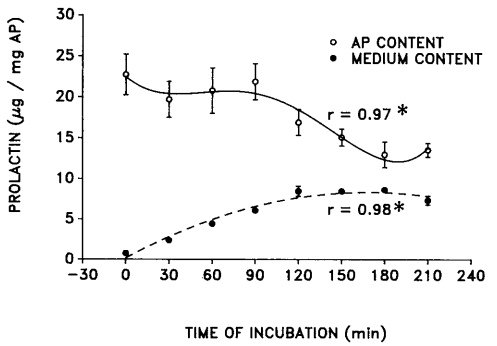

FIG. 1. Prolactin content of and concurrent cumulative prolactin release from pituitaries of ovariectomized and estrogen (PEP)-treated Fischer-344 rats. Pituitary fragments were incubated in static culture in the absence of dopamine. Each point represents mean ± SEM (n = 10). Twenty animals served as pituitary donors with each pituitary being cut into four fragments. The coefficients of determination (R^2) for the regressions were tested for significance by an F test. $^*P < 0.01$. The regressions were as follows: AP content = $22.5\text{-}0.15X + 4 \times 10^{-3}X^2 - 3 \times 10^{-5}X^3 + 8 \times 10^{-8}X^4$; medium content = $0.2 + 0.10X - 3 \times 10^{-4}X^2$, where X was minutes of incubation.

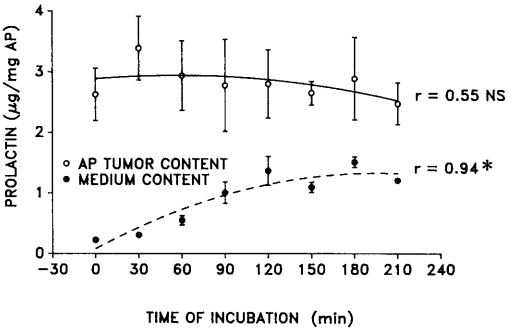

FIG. 2. Prolactin content of and concurrent cumulative prolactin release from anterior pituitary tumors of ovariectomized Fischer-344 rats implanted subcutaneously with a 5.0-mg pellet of diethylstibestrol for 8 weeks. Tumor fragments were incubated in a static culture in the absence of dopamine. Each point represents mean ± SEM (n = 8). Eight animals served as donors of the pituitary tumor tissue with eight fragments being obtained from each rat. Coefficients of determination (R^2) for the regressions were tested for significance by an F test. $^*P < 0.01$. NS = not significant. The regressions were as follows: tumor content = $2.9 + 2 \times 10^{-3}X - 2 \times 10^{-5}X^2$; medium content = $0.1 + 0.13X - 4 \times 10^{-5}X^2$, where X was minutes of incubation.

ovariectomized Wistar–Furth rats treated for 5 days with polyestradiol phosphate were incubated as above (Fig. 3). Prolactin was depleted from these pituitaries in a manner that best fit a fourth-order polynomial regression with approximately 20 μg/mg AP of PRL being depleted in the $3\frac{1}{2}$-hr incubation of which approximately 15 μg/mg AP was depleted in the first 30 min. Cumulative PRL release fit a second-order polynomial regression with approximately 12 μg/mg AP of PRL being recovered in the medium in the $3\frac{1}{2}$ hr. During the period of greatest depletion (0–30 min) 4 μ/mg AP was recovered in the medium.

In the last experiment transplanted MtTW15 anterior pituitary tumor tissue obtained from intact Wistar–Furth animals was incubated for 4 hr. Pituitary tumor PRL was depleted slightly over the incubation period (approximately 120 ng/mg AP tumor) and the data best fit a fourth-order polynomial regression (Fig. 4). Prolactin accumulating in the medium over the 4-hr incubation best fit a second-order polynomial regression with approximately 660 ng/mg AP tumor recovered over the incubation period. When the *in*

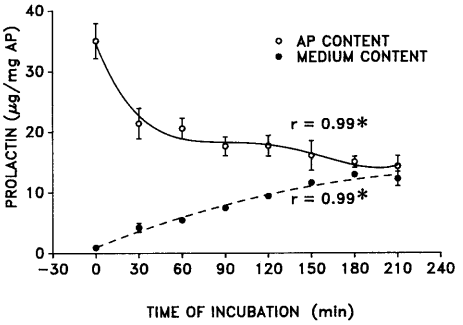

FIG. 3. Prolactin content of and concurrent cumulative prolactin release from pituitaries of ovariectomized and estrogen (PEP)-treated Wistar–Furth rats. Pituitary fragments were incubated in a static culture in the absence of dopamine. Each point represents mean ± SEM (n = 10). Twenty rats served as pituitary donor with each pituitary being cut into four fragments. The coefficients of determination (R^2) for the regressions were tested for significance by an F test. $^*P < 0.01$. The regressions were as follows: AP content = $34.7 - 0.61X + 8 \times 10^{-3}X^2 - 5 \times 10^{-5}X^3 + 9 \times 10^{-8}X^4$; medium content = $1.0 + 0.09X - 2 \times 10^{-4}X^2$, where X was minutes of incubation.

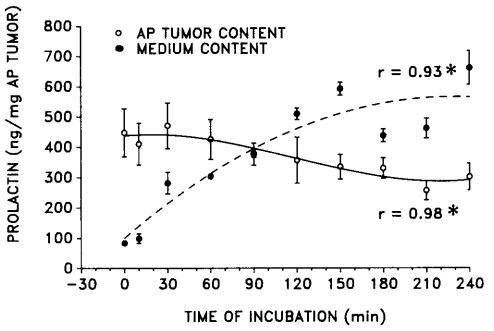

FIG. 4. Prolactin content of and concurrent cumulative prolactin release from MtTW15 pituitary tumor maintained subcutaneously for 4 weeks in intact female Wistar–Furth rats. Fragments of the tumors were incubated in a static culture in the absence of dopamine. Each point represents mean ± SEM (n = 8). Eight animals served as donors of the MtTW15 tumor fragments with nine fragments being obtained from each rat. The coefficients of determination (R^2) for the regressions were tested for significance by an F test. *$P < 0.01$. The regressions were as follows: tumor content = $0.4 + 6 \times 10^{-4}X - 2 \times 10^{-5}X^2 + 6 \times 10^{-8}X^3 - 4 \times 10^{-11}X^4$; medium content = $0.1 + 4 \times 10^{-3}X - 9 \times 10^{-5}X^2$, where X was minutes of incubation.

situ pituitaries of these same tumor-bearing rats were incubated, the pattern of depletion was like that of the ovariectomized estrogen-treated Wistar–Furth rats (Fig. 5) except that the initial PRL content was much less (14 ± 1.5 vs 35 ± 3). The pituitary depletion data appeared to fit a third-order polynomial regression but the limited number of points precluded the statistical verification of this. Pituitary depletion over the entire incubation was on the order of 6.8 μg/mg of AP with 4.4 μg/mg being depleted in the first 30 min. Accumulation of PRL in the medium best fit a second-order polynomial regression with 4.2 μg/mg AP being recovered over the 4-hr period of which 1.3 μg/mg AP was recovered in the first 30 min.

Discussion. The results of this study indicate that the process known as transformation (defined in this study as pituitary PRL depletion that could not be accounted for by the PRL recovered in the medium) is present in normal pituitaries from Fischer-344 and Wistar–Furth female rats treated with estrogen for 5 days but not in *in situ* pituitary tumors in DES-treated Fischer-344 rats or in MtTW15 pituitary tumors transplanted into intact Wistar–Furth rats.

To our knowledge, the observation of depletion without concurrent release has not been reported previously for pituitaries from estrogen-treated ovariectomized rats although such *in vitro* observations have been made using pituitaries from lactating rats by Nicoll (1) and Mena *et al.* (3). An early report by Swearingen (6) clearly indicated that more than one pool of prolactin existed in the pituitary of estrogen-treated ovariectomized rats as reflected by a discrepancy between the recovery of labeled PRL in the media compared with that lost from the pituitary tissue. She showed that the recovery of labeled PRL in medium exceeded that depleted from tissue and interpreted this to mean that a pool (or pools) of newly synthesized prolactin was undetectable by disc gel electrophoresis until it was released into medium. Such an interpretation is reasonably consistent with the concept that pituitary PRL is transformed prior to release to a form that is undetectable (hence the observation of

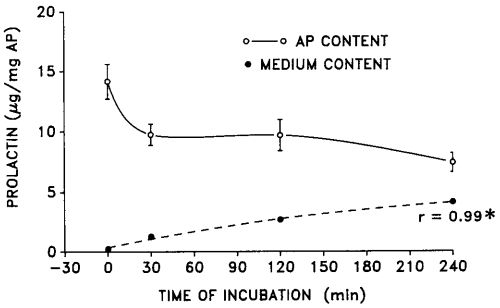

FIG. 5. Prolactin content of and concurrent cumulative prolactin release from pituitaries of intact female Wistar–Furth rats bearing subcutaneous transplants of MtTW15 anterior pituitary tumor for 4 weeks. Pituitary fragments were incubated in a static culture in the absence of dopamine. Each point represents mean ± SEM (n = 8). Eight rats served as donors of four pituitary fragments each. These rats were the same animals as in Fig. 4. The significance of the coefficient of determination for the regression was determined by an F test. *$P < 0.01$. The best fit for the tissue content appeared to be a third-order polynomial but this could not be verified statistically because of the limited number of points. The regression for medium content was $0.4 + 0.02X - 3 \times 10^{-5}X^2$.

depletion). However, recent data from Mena's labortory (4) indicates that the pool of PRL that transforms is not newly synthesized prolactin but PRL that has been stored in the cell for more than 1 hr but less than 16 hr.

That the time course of transformation was delayed in the Fischer-344 rats compared with Wistar–Furth rats (Fig. 1 vs Fig. 3) suggests that this mechanism may not always be as rapidly induced following removal from inhibitory hypothalamic tone as has been suggested (3). On the other hand, since transformation has been reported to occur repeatedly (3) it is possible that pituitary PRL from the Fischer-344 rats may have been rapidly transformed at the time of sacrifice and what was observed during the incubation was a second cycle of transformation. However, two arguments can be raised against this latter possibility. First, extreme care was exercised to ensure that transformation was not induced at sacrifice. We rapidly anesthetized all rats with ether prior to decapitation. This procedure has been shown to prevent transformation in lactating rats (11). In addition, the animals were not handled or disturbed prior to removal from their cages. Second, the Wistar–Furth rats did not show a marked second transformation under our *in vitro* conditions.

That transformation was not observed in either of the pituitary tumor models suggests that either the mechanism is not present or it is bypassed in hyperplastic or neoplastic cells. Previous reports have indicated that transformation is most evident with PRL that has been stored in the cell for more than 1 hr (4) and that rat pituitary tumor cells do not store an appreciable quantity of PRL (12). Our current results show the tumors contained less than 10% of the PRL present in respective normal pituitaries on a wet weight basis and this is consistent with the concept of a low storage pool. If transformation only occurs in PRL that has been stored, then it is reasonable to suspect that transformation may be bypassed in tumor cells with PRL being released quickly after synthesis. However, it is also possible that transformation was not present in the tumors due to the lack of an appropriate steroid milieu. We did not measure blood levels of steroids or DES at the time of sacrifice to verify that estrogenic steroid levels were elevated.

That transformation was present in the *in situ* pituitaries of Wistar–Furth rats that carried the MtTW15 tumor subcutaneously indicates that the hyperprolactinemia for 4 weeks did not inhibit transformation. However, it is important to note that the pituitary content was considerably less than that seen in the estrogen-treated Wistar–Furth rat. This decrese was probably due to the autofeedback effects of the high serum prolactin on AP conent in the tumor-bearing rats (13).

We conclude from these experiments that rat pituitary tumors do not exhibit prolactin transformation. This may be useful in understanding the exact mechanism of this process and the nature of prolactin processing within normal and hyperplastic or neoplastic lactotrophs.

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