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Secretion of Human Prolactin *In Vitro*[1] (39258)

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(Introduced by P. M. Gullino)

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Prolactin is a phylogenetically ancient hormone whose effects extend throughout many of the organ systems of man, but whose physiologic significance, other than in lactation, remains unclear. This polypeptide hormone has come under close scrutiny during the past two decades because of its apparent role in the pathophysiology of human breast cancer. Investigations on the physiology and pathophysiology of prolactin require both a sizable supply of hormone for *in vivo* and *in vitro* studies and the methodology for direct study of the control of its synthesis and secretion. Several problems, however, have curtailed this work. Supplies of human prolactin (hPRL) have been limited since a single pituitary gland contains only 250 μg of the hormone, and the number of pituitaries available for extraction is diminishing because fewer autopsies are now being performed in the United States. Direct studies of human prolactin synthesis and secretion under physiologic or pharmacologic stimuli are difficult to perform in humans; *in vitro* techniques are often cumbersome and may not accurately reflect *in vivo* behavior.

The artificial capillary culture technique (1) has been shown to provide an *in vitro* milieu that closely simulates the *in vivo* situation (2) and is capable of producing large quantities of hormones *in vitro* (3). The following studies were performed to demonstrate that large quantities of human prolactin can be produced and that the secretory dynamics can be followed, in near physiologic conditions, by using the artificial capillary culture system.

Materials and methods. The perfusion circuit consists of a capillary culture unit (CCU) fed by a roller pump with medium from a reservoir. All components were connected by silastic tubing which also serves as an oxygenator and pCO_2 controller (Fig. 1).

The capillary culture units (CCU) for both cell types were prepared as described previously (1) with the following modifications. An 8 × 1 cm polycarbonate tube with tapered ends and two loading side ports served as the shell. Eight-four cellulose acetate capillaries, 200 μm i.d. × 250 μm o.d. (Dow Chemical Co., Walnut Creek, Calif.), nominally permeable to substances up to 100,000 mol wt, were randomly dispersed with 90 silicone polycarbonate capillaries (Dow Chemical Co.) having the same dimensions but permeable only to gases. Both ends of the mixed capillary bundle were potted into the shell ends with catalyzed silicone rubber monomer and allowed to harden overnight. The excess potting material was trimmed flush to the shell ends, exposing the patent capillaries and allowing fluid supplied to one end to pass only through the capillaries without inducing bulk flow within the extracapillary space. Ground-glass female tapered fittings allowed insertion of the CCU into the perfusion circuit.

Each CCU was sterilized in ethylene oxide for 10 hr, aired for 1-2 days, and inserted into the previously steam-autoclaved circuit. The perfusion circuit was arranged in either a recirculating or single-pass mode by clamping the Silastic connecting tubing (Dow Corning Co.) at the appropriate points. The circuit was then flushed with 10% ethanol in water for 2 days followed by two changes of sterile deionized water; the reservoir was then refilled with complete nutrient medium, and the entire circuit was placed in a humidified 5% CO_2-air incubator at 37°. Cells were injected through the shell ports and settled onto the capillaries to receive metabolic support from the perfus-

[1] Portions of this research were presented at the Third International Symposium on Growth Hormone and Related Peptides, Milan, Italy, September 17-20, 1975.

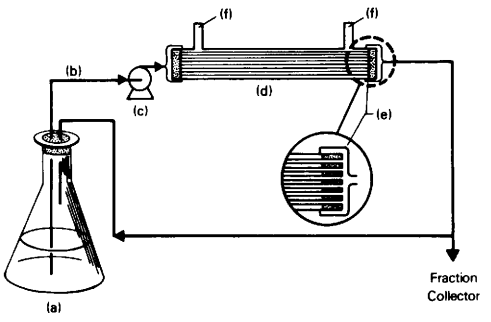

FIG. 1. The perfusion circuit consists of a reservoir (a), connecting tubing (b), roller pump (c), and capillary culture unit (d). Medium pumped to the capillary culture unit passes through the capillaries only (e). Cells are injected into the extracapillary space through loading ports (f).

ate by diffusion through the capillary walls. In the recirculating mode, medium was replaced every 1–4 days and saved for analysis, whereas when the single pass mode was employed, fresh medium was always used and the perfusate was saved in a fraction collector as it exited the CCU.

Aliquots of tissue culture medium were assayed for human prolactin by specific double-antibody radioimmunoassay. Human prolactin (hPRL) standard V-L-S-2, obtained by extraction from human pituitaries, was a gift from the Hormone Distribution Program, NIAMDD. The [^{125}I]hPRL was made by a modification (4) of the lactoperoxidase technique. Human prolactin antisera RB-4 was provided by Dr. Judith Vaitukaitis, NICHD. Radioimmunoassay data was processed by the computer programs and methods of Rodbard (5).

Results. Pituitary tumor cells were obtained from a 51-year-old male (RUS) having a 3-yr history of progressive visual field cuts, an enlarged sella, and high levels of serum prolactin. Approximately 300 mg of pituitary tumor fragments, obtained via a transfrontal approach, were immediately placed in Ham's F-10 tissue culture medium[2] containing 100 mg% crude collagenase (Worthington Biochemical Corp., Freehold, N.J.). The suspension was stirred gently at 37° for 30 min in a 5% CO_2–air incubator, centrifuged at 100*g* for 5 min, and resuspended in 3 ml of complete medium. One CCU containing these RUS cells, maintained in the recirculating mode, produced a total of 3 mg of hPRL during the first 4 months of culture. The rate of secretion decreased steadily during the entire culture period (Fig. 2).

Approximately 100 mg of pituitary tumor fragments were obtained from a 50-year-old male (MCG) with galactorrhea, gynecomastia, and enlarged sella, and kept in Ham's F-10 medium at room temperature for 18 hr. The tissue was then gently dispersed by pipetting, centrifuged at 100*g* for 5 min, and resuspended in 2 ml of complete medium. Less than 50 μg of this tissue was placed in the extracapillary space of another CCU arranged in the recirculating mode. The daily hPRL secretory rate declined from an initial value of 15 μg to 5 μg by Day 18. On Day 4 the medium was replaced with fresh medium, the single pass mode of circulation (Fig. 1) was implemented, and the immediate secretory rates were followed. The medium was collected at 1-min intervals, stored at −20°, and subsequently thawed for radioimmunoassay. The basal rate of secretion of the tumor cells was 15 ng/min. A step change was then induced by replacing the nutrient medium for 1 hr with a solution of complete medium plus 100-ng thyrotropic releasing hormone (TRH) (gift from Abbott Laboratories, North Chicago, Ill.) and 25 μCi of [^{14}C]protein hydrolysate (reconstituted Schwarz/Mann, algal profile)/ml. Appearance of ^{14}C in the effluent heralded the presence of TRH and allowed observation of the temporal relationship between stimulation and hPRL release. Figure 3 shows that an 8-min lag occurred between the exposure of the pituitary tissue to TRH and the increased secretion of hPRL. The massive burst of hPRL secretion was followed by a rapid decline in secretory rate and a second peak of secretion. The secretory rate gradually decreased over the next 3 hr to near-baseline levels. After an initial TRH stimulation, subsequent exposures of the same culture to TRH did not cause the rapid release phase; however, a gradual late rise to twice the basal secretory rate occurred, followed by a gradual return to the basal rate.

Discussion. Significant amounts of human

[2] Ham's F-10 with 13.5% horse serum (Gibco, Grand Island Biological Co., Grand Island, N.Y.), 3.2% fetal calf serum (Gibco), 50 μg streptomycin/ml, and 50 units of penicillin/ml.

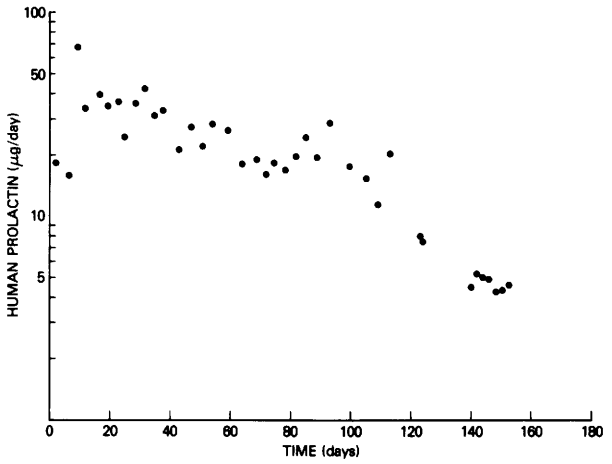

FIG. 2. RUS pituitary cells perfused in the recirculating mode produced more than 3 mg of hPRL during the first 4 months of culture.

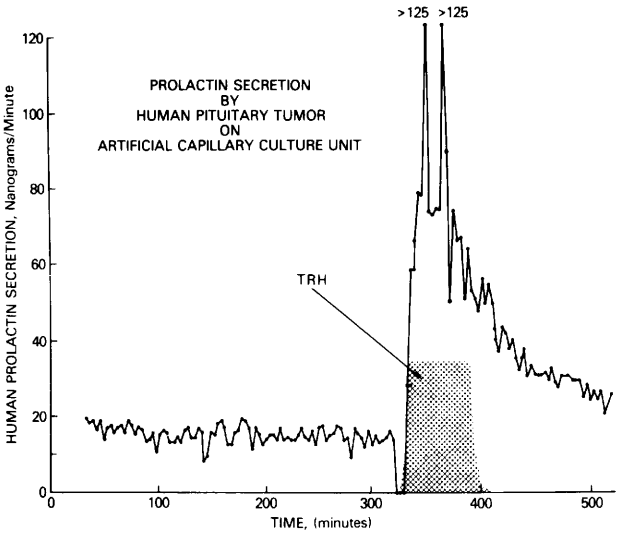

FIG. 3. MCG pituitary cells secreted 15 ng of hPRL into the medium/min in the basal state. Within 8 min after exposure to TRH, a massive amount of hPRL was released followed by a gradual decline toward the basal secretion rate.

prolactin may now be produced in a relatively straightforward manner. The 3 mg of prolactin obtained from a single CCU inoculated with relatively few cells is manyfold greater than the amount extractable from a single human pituitary gland. Since the capillary membrane is permeable only to substances of relatively low molecular weight, contamination by viral particles is avoided. Culture units of larger size could provide far greater amounts of hormones for biochemical, biological, and clinical studies.

The capillary culture technique provides cells with an artificially vascularized environment which closely simulates the *in vivo* state allowing transient cellular responses to both physiologic and pharmacologic agents to be followed. One such control of hPRL release is TRH (6, 7). Jacobs *et al.* (8) showed that 15 min after an iv bolus of TRH was injected into human volunteers, there was a marked increase in circulating prolactin followed by a gradual fall to baseline levels within 3 hr. Thyrotropic releasing hormone infusion into isolated hypophyseal arteries of rats gave a similar response (9).

In vitro studies by Tashjian and co-workers (10, 11), however, showed a gradual increase in prolactin secretion when monolayers of established rat pituitary cells were exposed to TRH. This reflected increased prolactin synthesis, beginning 20 min after exposure to TRH, followed 40 min later by increased prolactin secretion. No immediate release of stored prolactin was observed with these cell lines.

The response of the primary human pituitary tumor cultured within the capillary unit duplicates the physiologic response observed *in vivo*. TRH has a low molecular weight enabling it to diffuse very rapidly through the capillary wall. This indicates that the 8-min lag period prior to hPRL release is not due to slow penetration of the extracapillary space, but rather to reflection of the time required by the cell to effect the release phenomenon. The prolonged hPRL secretion may be due to TRH remaining bound to the pituitary cells with persistence of accelerated release and/or an elevated rate of hPRL synthesis by the stimulated cells. The immediate and massive release of stored prolactin occurred only with the first exposure to TRH; repeated stimulation thereafter showed only a slight and gradual increase in prolactin secretion without subsequent changes in basal secretory rates. This implies that other factors, perhaps hypothalamic, may be necessary for prolactin to be stored in forms which can be released rapidly. The elevated but gradually decreasing rate of prolactin secretion, 30 min after TRH stimulation, may reflect the double pool suggested previously (12) and, thus, be similar to the double pool of stored insulin in the pancreatic beta cell (13). This may be a generalized response of the endocrine cell to prolonged physiologic stimulation.

Summary. Cells from two human pituitary tumors were grown in capillary culture units and prolactin production was measured. A single unit could produce 3 mg of prolactin over a 4-month period. The cultured cells responded to TRH exposure by increasing their rate of prolactin secretion. Cultivation of cells in capillary units could be the method of choice for reducing the shortage of human hormones.

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