OBSERVATION OF PRODUCTION OF IMMUNOACTIVE PROLACTIN BY NORMAL HUMAN CONNECTIVE TISSUE IN CELL CULTURE

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SUMMARY

Data from our in vitro studies indicate a new source of prolactin (PRL)-like activity, normal human connective tissue. Fascial cells from primary culture and subsequent passages produced an extracellular antigen which specifically reacted in a radioimmunoassay RIA developed to detect human pituitary PRL. An initial peak or first surge of fascial PRL-like activity occurred between 4 and 15 d in primary culture. Ibuprofen, cytotoxic levels of 0.01% azide, or 7.5 mM EDTA and medium lacking serum [fetal bovine serum (FBS)] significantly \( P < 0.05 \) reduced PRL-like activity levels, whereas female steroids, 257 to 342 milliosmolarity, 1 to 3.6 mg/ml glucose, 2 to 20% FBS, and dialyzed FBS (MWCO \( \sim \)1 kDa) were without effect. Optimum production of PRL-like activity occurred at pH 7.3. A second surge began after 18 d and continued until passage indicating that perhaps two populations of cells produced PRL-like activity in primary culture. Production of PRL-like activity by cells from early passages (1 and 2) became detectable at confluence, was serum-dependent, showed two patterns (tonic, rising to plateau), and averaged 3.2 fg·cell\(^{-1}\)·3 d\(^{-1}\) feed interval. Cells from late passages showed morphologic damage from repetitive trypsinization, aging, and reduced production of PRL-like activity with aberrant production pattern. Production of PRL-like activity was maintained in an unusual long-term culture. These in vitro studies demonstrate the most recently recognized and ubiquitous source of human extrapituitary PRL or PRL-like activity, normal connective tissue (fascia).

Key words: extrapituitary prolactinlike activity; connective tissue; fascia; fibroblasts.

INTRODUCTION

Utilizing radioimmunoassay (RIA) technology and, as standard and tracer, purified human prolactin (PRL) isolated from the pituitary gland, several extrapituitary sources of PRL have been reported in humans (1-12). These included endometrium in women during the mid-to-late luteal phase of the menstrual cycle, decidualized endometrium throughout pregnancy, decidualized tissue of tubal pregnancy, leiomyomas derived from normal myometrium, normal myometrium, and the focus of this paper, connective tissue, specifically, fascia. Our working hypothesis derived from these data stated that extrapituitary PRL production was a general property of mesenchymal tissue which served autocrine and paracrine roles at local tissue sites (13). Most recently, PRL was shown to be produced by a human B-lymphoblastoid cell line, IM-9-P (14). Fascial PRL detected in conditioned medium from cultured normal connective tissue possessed properties of pituitary PRL: 1) immunochemical antigenic reactivity and immunoparallelism by RIA, b) physicochemical comparable elution profile in the fractionation range of gel filtration column chromatography, and c) functional lactogen activity in the Nb2 lymphoma cell bioassay (12,15). Because we currently lack the definitive evidence, amino acid sequence data, proving fascial and pituitary PRL are identical, the term prolactinlike activity will be used here when referring to the former molecule. This study was conducted to observe and characterize the production of fascial PRL-like activity in cell culture. The findings are reported for this most ubiquitous and provocative source of extrapituitary and extrauterine PRL or PRL-like activity thus far discovered in humans.

MATERIALS AND METHODS

Materials

A 10x concentrate of Dulbecco's phosphate buffered saline without calcium and magnesium, 2.5% solution of...
trypsin, 10× concentrate of Earle’s balanced salt solution, minimum essential medium (MEM) 100X vitamins, MEM 50X essential amino acids, MEM 100X nonessential amino acids, and heat-inactivated fetal bovine serum (FBS) were purchased from GIBCO Laboratories, Chagrin Falls, OH. Nystatin, ibuprofen, estradiol, and progesterone were obtained at Sigma Chemical Company, St. Louis, MO. Penicillin-streptomycin mixture 17-719R was bought from Whittaker M. A. Bioproducts, Walkersville, MD. Disposable Millex-GS 0.22-μm filtration units SLGS0250S were purchased at Millipore Corporation, Bedford, MA. The tracer [125I]prolactin coded NEX-127 was provided by NEN Research Products, Boston, MA.

Connective Tissue

Rectus fascia (200 to 300 mg) obtained from below the level of the umbilicus through either midline or Pfannenstiel’s incision was aseptically and freshly collected at surgery from gynecologic patients in accordance with formal consent policy of the Human Experimentation Committee. Tissue was transported in 5 ml of culture medium at 23 °C and established in primary culture within 1.5 h.

Cell Culture

Primary. Fascia freed of extraneous adipose tissue was scalpel-minced into 1-mm³ pieces in 3 ml of fresh medium at 23 °C in 60 × 15-mm plastic petri dishes (Falcon 3002, Becton Dickinson & Co., Oxnard, CA). Between five and eight pieces per well were distributed into 3 × 14-mm wells in six-multiwell tissue culture plates (Falcon 3046, Becton Dickinson). Wells were fed 1 ml of fresh medium and incubated at 37 °C in an atmosphere of 98% humidity and 5% CO₂:95% air.

Passage. Confluent cells from primary cultures or passages were washed twice with 1 ml of physiologic Dulbecco’s phosphate buffered saline lacking calcium and magnesium, and released from the culture surface with 0.25 to 0.5 ml of 0.16% trypsin in saline by incubating for 10 to 30 min at 37 °C in an atmosphere of 98% humidity and 5% CO₂:95% air. Cells were triturated 4 times, diluted with 3 ml of fresh medium and split 1:3 by evenly dispensing into three wells. Large pieces of primary tissue were excluded from passage by trapping on nylon membrane with 150- to 160-μm square mesh (Nydex).

Cell counts. Trypsin-dispersed cells were appropriately diluted with Turk’s solution, a fixative and stain, and using light microscopy were manually counted in a hemacytometer by averaging the four outer squares.

Medium

One liter of physiologic Earle’s balanced salt solution contained 20 ml of a 100X solution of MEM vitamins, 40 ml of a 50X solution of MEM essential amino acids and 20 ml of a 100X solution of MEM nonessential amino acids, 4 mM L-glutamine, 1 mM sodium pyruvate, 125 U/ml potassium penicillin G, 125 μg/ml streptomycin sulfate, and 100 U/ml nystatin. This basic medium, equilibrated in an atmosphere of 98% humidity and 5% CO₂:95% air at 37 °C, was adjusted to pH 7.3 to 7.4 with 1N NaOH or HCl and to 285 mOsm with sterile, pyrogen-free water (Travenol). The standard 20% serum-supplemented medium was prepared by mixing 4 vol basic medium with 1 vol FBS. Medium was filtered through a 0.22-μm membrane and refrigerated at +1 °C. The lot of FBS used in these studies contained 0.1 ng/ml PRL.

Test Medium

Test media were prepared by modification of the standard medium. Osmolarity was adjusted with either water or an aqueous solution of 1.45 M sodium chloride and 50 mM potassium chloride. The pH was adjusted with minimal volumes of sterile 2N hydrochloric acid or sodium hydroxide. Fetal bovine serum was dialyzed at 2 °C for 8 to 16 h in cellulose nitrate tubing (MWCO ~1 kDa) against 9 vol of serum-free medium or physiologic saline with one change of dialytic solution.

Test Reagents

Stock solutions of 50 mg/ml ibuprofen, 115 μg/ml estradiol (E₂), 0.6 mg/ml progesterone (P), and a mixture of E₂ and P were dissolved in 100% ethanol, stored at 2 °C and diluted in medium for use. Stock aqueous solutions of 2% sodium azide and 0.15 M disodium EDTA, pH 7.4, were stored at 23 °C and added to medium just before use.

FIG. 1. Prolactin-like activity produced by normal human connective tissue, fascia, during the first 6 d in primary culture. Antigen concentration in conditioned medium was quantified by RIA specific for human pituitary PRL. The values, mean ± SD, represented by bars and T-bars, respectively, of triplicate cultures are a) total PRL-like activity, solid bar; and b) "endogenous" activity released from control tissue wells after treatment throughout the culture period with 0.01 to 0.02% sodium azide or 7.5 mM EDTA, open bar. Two (7%) of 29 individual tissues did not produce detectable antigen.