Paracrine Stimulation of Polarized Secretion from Monolayers of a Neoplastic Prostatic Epithelial Cell Line by Prostatic Stromal Cell Proteins

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ABSTRACT

The paracrine influence of prostatic stromal cell proteins on a neoplastic prostate cell line (PA-III) was investigated. We have utilized an in vitro experimental model whereby confluent epithelial sheets of PA-III cells are grown on Matrigel-coated filters in bicameral chambers (Millicell-HA). Confluence of the epithelial sheet was confirmed morphologically by electrical resistance measurements and by impedance of [3H]inulin permeability across paracellular channels. Stromal cells were isolated from the ventral prostate of 50-day-old rats by isopycnic Percoll centrifugation. Purity (92%) of the isolated stromal cells was confirmed by indirect immunofluorescence of vimentin intermediate filaments. Prostatic epithelial cells were negative for vimentin immunofluorescence. Prostatic stromal cell secretory proteins with molecular weights >10,000 were placed in the basal reservoir of the bicameral chambers underneath the confluent epithelial sheets of PA-III cells in a manner that mimics the relationship between stroma and epithelium in vivo. After 24 h incubation the stromal cell proteins increased the [35S]methionine-labeled protein secretion from the epithelial sheet of cells. Trypsinization of the stromal cell secretory proteins eliminated the stimulatory effect on epithelial protein secretion. Two-dimensional gel electrophoresis of the [35S]methionine-labeled epithelial protein secretion showed that the stromal cell proteins induced the secretion of a novel peptide (SE-1) from the basal domain of the epithelial sheet of cells within the first hour of metabolic labeling. These results indicate that stromal cell secretory proteins contain a stimulatory protein that can induce overall protein secretion as well as the vectorial secretion of a novel peptide from the basal domain of PA-III epithelial cells. These results are consistent with a paracrine interaction between epithelial and stromal cells in the regulation of prostatic secretion.

INTRODUCTION

The testosterone dependence of the prostate gland for the development and maintenance of differentiated structure and secretory function has long been recognized as a major mechanism for the endocrine regulation of prostatic growth (1). In addition to the global effects of testosterone, it has become increasingly clear that benign tumors are not the only indication of aberrant prostatic growth. In this context, paracrine regulation of prostatic growth was first suggested by Franks et al. (2) after observing a lack of growth capacity of epithelium which had been separated from its stroma. Subsequently, the elegant experiments of Cunha et al. (3, 4) demonstrated that fetal mesenchyme (stroma) induced prostatic epithelial morphogenesis from urothelium. Indeed, increased amounts of mesenchyme relative to epithelium increased total prostatic growth (5), suggesting a dose-dependent regulation of cellular proliferation. Prostatic stroma and epithelia respond differentially to androgenic deprivation and stimulation (6). During development, androgenic binding sites are restricted to the mesenchyme, indicating that morphogenesis entails an androgen-dependent proliferation of stromal cells which in turn mediate epithelial proliferation and development within the acini of the prostate (7). In addition, the proliferation of epithelial cell growth within the prostatic acini exhibits a regional heterogeneity, further indicating that local control mechanisms, presumably through paracrine interactions with the stroma, regulate differentiation and development (8, 9). Implicit in the concept of stroma inducing, specifying, and maintaining epithelial phenotype and functional activity is that disturbance of stromal-epithelial interactions may lead to unregulated aberrant growth. In this context, McNeal (10) suggested that benign prostatic hyperplasia in adults may result from reversion of stroma to an embryonic state leading to an aberrant stimulation of epithelial cell proliferation. Hence, detailed analysis of paracrine regulated growth may eventually allow the development of strategies to modify and manipulate aberrant prostatic proliferation.

By virtue of tight junctions between the apicolateral plasma membranes of adjacent epithelial cells, prostatic acini are compartmentalized and bathed in two distinct microenvironments, one apical (luminal) and the other basal. The proximal microenvironment contains a variety of epithelial secretory products including proteins such as prostatic (11), a sperm-binding protein (12), a non-steroid-binding protein with a molecular weight of 22,000 (13, 14), and a steroid-binding sialoglycoprotein (15). The basal surface of the epithelial cells rests on a semipermeable basement membrane through which basally secreted epithelial cell products may permeate and in the opposite direction blood-borne constituents and stromal cell paracrine factors may also mix and permeate to form the basal milieu. In order to study paracrine interactions between the basal surface of epithelium and underlying stroma, as normally occurs in vivo, we have adapted an in vitro experimental system (16-18) devised to study other reproductive tract epithelia (seminiferous epithelium, epididymis) to that of the prostate. In this report we characterize the growth of confluent epithelial sheets of a neoplastic prostate cell line (19) in bicameral chambers and report paracrine interactions between epithelial sheets of the cell line and stromal cell secretory proteins derived from prostatic primary cultures of young adult (50-day-old) rats.

MATERIALS AND METHODS

Culture of Prostate Epithelial Cells. A Lobund Wistar rat epithelial adenocarcinoma cell line (PA-III) was a kind gift from Dr. M. Pollard (19). PA-III cells were propagated in 225-ml plastic culture flasks (Becton Dickinson Company, Lincoln Park, NJ) containing heat-inactivated 5% FCS (Hyclone Laboratories Inc., Logan, UT), DMEM/Ham's F-12/Hepes buffer (Irvine Scientific, Santa Ana, CA), 2 mM glutamine (Sigma Chemical Co., St. Louis, MO), 100 units/ml penicillin (Sigma), 100 µg/ml streptomycin (Sigma), and 10⁻⁷ M testosterone. The abbreviations used are: FCS, fetal calf serum; DMEM, Dulbecco's minimum essential medium; Hepes, 4(2-hydroxyethyl)-1-piperazineneethanesulfonic acid; SFDM, serum-free defined medium; PBS, phosphate-buffered saline; SCCM, stromal cell-conditioned medium.
(Sigma). All cultures were maintained in a humidified incubator at 37°C in 5% CO2/air. Culture media was changed every third day. Subsequently, PA-III cells were removed from the culture flasks with 5 mg/ml trypsin and 2 mg/ml EDTA in balanced salt solution without calcium and magnesium (Whittaker Bioproducts, Walkersville, MD). The resulting single-cell suspension of PA-III cells was washed by centrifugation at 200 x g in culture media and 5 x 10^5 cells seeded on basement membrane Matrigel (Collaborative Research Inc, Lexington, MA) coated Millipore filters (0.45-μm pore size) in bicameral (Millicell-HA) dual compartment chambers (Millipore Corp., Bedford, MA). A schematic diagram of epithelial cells cultured in the bicameral chambers is shown is Fig. 1. PA-III cells grown in the bicameral chambers were cultured in either 5% FCS (as above) or a SFDM consisting of 2 μg/ml zinc-free insulin (Collaborative Research), 10 ng/ml epidermal growth factor (Collaborative Research), 1 μg/ml transferrin (Sigma), 10^-7 M hydrocortisone (Sigma), 2 mM glutamine (Sigma), and 10^-7 M testosterone (Sigma) formulated in DMEM/Ham’s F-12/Hepes (Irvine Scientific) containing 100 units/ml penicillin (Sigma) and 100 μg/ml streptomycin (Sigma).

Characterization of Confluence. Electrical resistance to the passage of current across the monolayers of epithelial cells grown in the bicameral chambers was measured using an epithelial voltohmmeter (World Precision Instruments Inc., New Haven, CT) as an indication of the establishment of transepithelial permeability barriers, as previously described (17). Background electrical resistance measurements on Matrigel-coated filters in the absence of PA-III cells were subtracted from measurements of the epithelial cell resistance.

[3H]Inulin permeability across Matrigel-coated filters in the absence or presence of PA-III cells was determined by placing 1 x 10^6 cpm of [3H]Inulin (ICN Radiochemicals, Irvine, CA) in the basolateral (bicameral) chambers. Subsequently, over a 6-h period, an aliquot of culture media from the apical reservoir was analyzed for the content of [3H]Inulin in a liquid scintillation counter (Beckmann LS 8000; Wakefield, MA).

Morphology of PA-III cells grown in bicameral chambers was assessed by electron microscopy as previously described (20). Confluent growth of PA-III cells in the bicameral chambers was confirmed by staining some of the epithelial sheets that had been fixed for electron microscopy with 4.8 g/liter Harris’ alum hematoxylin (Accra Lab, Bridgeport, NJ), followed by rinsing in PBS, substitution with a graded series of ethanol, and clearing the cells in xylene. The epithelial sheets were then attached to microscope slides with tissue tack (Polysciences, Warrington, PA) and mounted in Flo-texx (Lerner Labs, Stanford, CT) for observation and photography.

Isolation of Stromal Cells and Preparation of Conditioned Media. The ventral prostate from 50-day-old Wistar rats that had been carbon dioxide asphyxiated were cleaned of associated adipose tissue and minced into small (1-3 mm³) blocks of tissue with a pair of fine scissors. The resulting single-cell suspension of PA-III cells was digested in lN NaOH and labeled PA-III epithelial cell secretory proteins were collected over a period of 24 h. The epithelial cells were digested in 1 N HCl and neutralized with an equal volume of 1 N HCl. Subsequently, secreted and intracellular [35S]methionine-labeled protein was precipitated with TCA (65). For the dose-dependent studies PA-III cells were incubated with 3, 15, and 30 μg/ml SCCM over 24 h. In order to degrade protein, an aliquot of the 30 μg/ml SCCM was trypsinized and subsequently neutralized with soybean trypsin inhibitor according to the method of

PARACRINE STIMULATION OF PROSTATE SECRETION

PROSTATE BICAMERAL CULTURE CHAMBER SYSTEM

Fig. 1. A schematic diagram of a bicameral culture chamber used to grow PA-III epithelial cells at confluence on a Millipore filter coated with extracellular matrix. By virtue of the confluent epithelial sheet of cells culture media in the apical and basal reservoirs are prevented from mixing, thus compartmentalizing the chambers. Stromal cells or their conditioned media are included in the basal reservoir in a spatial relationship comparable to their in vivo location.

1967
Djakiew and Dym (22). For the pulse-chase studies PA-III cells were initially incubated for 24 h with or without 30 μg/ml SCCM. Subsequently, the cells were pulsed with 1.5 mCi/ml [35S]methionine for 20 min and chased with a 1000-fold excess of [35S]methionine. All collected protein samples were made 2 mM with phenylmethylsulfonyl fluoride, and the secretory proteins were centrifuged at 10,000 x g for 30 min. Except for the time course studies, unincorporated [35S]methionine was removed from the supernatent of the samples by repeated dilution (8 times) in distilled water followed by centrifugation of the metabolically labeled proteins in Centriprep-10 microconcentrators (Amicon). Total [35S]methionine-incorporated activity was determined by resuspending the Centriprep-10 retained material in distilled water to its original volume, or the trichloroacetic acid precipitate in 100 μl 0.1 N NaOH, and measuring [35S]methionine activity in a liquid scintillation counter (Beckmann). The filters on which the epithelial cells were growing were removed from the bicameral chambers, and the DNA content of the cells was estimated according to the method of Burton (23). For the time course studies DNA was estimated from parallel cultures similarly treated with or without SCCM. Total DNA/bicameral chamber was converted to cell number using a previously determined estimate of 13.0 ± 0.4 μg (SEM) DNA/10⁶ PA-III epithelial cells. All estimates of [35S]methionine-labeled protein secretion were normalized to the total number of PA-III cells in each bicameral chamber.

Electrophoresis and Fluorography. Protein samples were prepared for two-dimensional polyacrylamide gel electrophoresis according to the method of O’Farrell (24) as modified by Djakiew and Dym (22). Briefly, protein samples were diluted in an equal volume of nonreducing sample buffer and heated to 100°C for 90 s. Equal cpm of protein samples were separated on 4% polyacrylamide tube gels (3 mm inside diameter) by isoelectric focusing over 6900 V·h. Proteins were separated in the second dimension on polyacrylamide slab gels with 10% stacking gels and 10% separating gels. Standard peptides for determining isoelectric point in the first dimension and molecular weight in the second dimension were obtained from Bio-Rad and New England Nuclear (Boston, MA), respectively. Fixed gels were impregnated with fluor by soaking twice in dimethyl sulfoxide for 30 min each, followed by 3 h in 22% PPO/dimethyl sulfoxide and washing in distilled water for 1 h. Gels were dried under vacuum at 50°C and exposed for autoradiography to X-Omat AR diagnostic film (Kodak, Rochester, NY) for 4 days at −70°C.

Statistical Analysis. The statistical significance of differences between treatments was tested by analysis of variance using the Newman-Keuls multiple range test and by Student’s t test. The SEM values that are given as estimates of the dispersion in the results were calculated for each treatment from estimates of the variance between replicates.

RESULTS

Characterization of Confluence of the Epithelial Cell Monolayers in the Bicameral Chambers. Fig. 2 shows a low power view of PA-III epithelial cells that have been removed from a bicameral chamber and stained with Harris’ alum hematoxyn. Note that the epithelial sheet of cells has grown to confluence over the surface of the filter. Fig. 3, A and B, show low power photographic montages of epithelial cells grown for 5 days to confluence on the filters in either SFDM or 5% FCS, respectively. The characterization of the PA-III adenocarcinoma cell line (19) and its ultrastructural characteristics (25) have been discussed in the literature, hence only pertinent features of these cells grown in the bicameral chambers will be described. In this context, it is noteworthy that, irrespective of the culture conditions, the epithelia were lined by short microvilli on their apical surface (Fig. 3, A and B) and were joined at their apical-lateral plasma membranes by tight junctions. In addition, the basal cytoplasm of the cells interdigitated into the pores of the Millipore filters thereby enhancing the basal surface area of the cells. The most pertinent morphological difference between epithelia grown under different culture conditions was that the cells grown in SFDM were columnar (Fig. 3A), whereas those cells grown in 5% FCS were relatively squamous (Fig. 3B).

Fig. 4 shows the electrical resistance of PA-III epithelial cells seeded in the bicameral chambers and cultured in either SFDM or 5% FCS. The electrical resistance of the forming epithelial sheets of PA-III cells differed significantly (P < 0.05) when grown in either SFDM or 5% FCS. Irrespective of the culture media, the peak electrical resistance of the epithelial sheets occurred on approximately the 5th day of culture. In the case of epithelia grown in SFDM, the electrical resistance remained relatively constant for an additional 7 days in culture, whereas the electrical resistance of epithelia grown in 5% FCS gradually declined over the following 7 days in culture.

Fig. 5 shows the passage of [3H]inulin across bicameral chambers (basal to apical) in the absence of epithelial cells or across confluent epithelial sheets of cells grown for 5 days in either SFDM or 5% FCS. The passage of [3H]inulin across the bicameral chamber in the absence of any cells was approximately 2.8% of the total added/h. In contrast, the rate of passage of [3H]inulin across a confluent culture of PA-III cells grown in 5% FCS was approximately 0.5% of the total added/h, whereas the rate of passage [3H]inulin across a confluent culture of PA-III cells grown in SFDM was approximately 0.23% of the total added/h.

Separation of Stromal Cells from Epithelial Cells. Figs. 6, A and B, show a phase contrast image and the corresponding indirect immunofluorescence of vimentin intermediate filaments, respectively, of a primary culture of stromal cells which have been separated on continuous isopyknic Percoll gradients and grown on glass coverslips. The stromal cells (Fig. 6A) consisted of attenuated and spindle-shaped cells. The cytoplasm of these cells contained numerous stress fibers and was deficient in vesicular inclusions. For comparison a primary culture of epithelial cells that had been similarly prepared is shown in Fig. 6, C and D. The phase contrast image of the epithelial cells (Fig. 6C) exhibited numerous prominent refractive vesicles in the cytoplasm. Note that vimentin intermediate filament immunofluorescence was not detected in the epithelial cell culture (Fig. 6D). Based on vimentin immunofluorescence the purity of the stromal cells isolated by isopyknic sedimentation was 92 ± 3% (n = 3).

Stimulation of PA-III Epithelial Cell Protein Secretion by Stromal Cell-conditioned Media. Figs. 7 and 8 show the time course of [35S]methionine incorporation into intracellular and extracellular proteins. The most pertinent morphological difference between epithelia grown under different culture conditions was that the cells grown in SFDM were columnar (Fig. 3A), whereas those cells grown in 5% FCS were relatively squamous (Fig. 3B).
secretory protein, respectively, of PA-III cells incubated with or without 30 μg/ml SCCM. [35S]Methionine incorporation into intracellular and secretory protein was saturated by 18 h for both the control and SCCM treatments. Whereas [35S]methionine incorporation into intracellular proteins was not significantly different between the two treatments (Fig. 7), SCCM stimulated (P < 0.05) protein secretion from the PA-III cells over the 24-h incubation period (Fig. 8). Fig. 9 shows that SCCM, placed in the basal reservoirs of bicameral chambers, significantly (P < 0.05) stimulated [35S]methionine-labeled protein secretion from the confluent epithelial sheets of PA-III cells in a dose-dependent relationship over a period of 24 h. When [35S]methionine-labeled protein secretion was expressed as a percentage relative to the control value (100% = 3231 cpm/10⁶ cells), trypsinization and subsequent neutralization of the 30-μg/ml SCCM™ treatment eliminated the stimulatory effect of the SCCM on PA-III epithelial protein secretion (98 ± 11%, n = 3). In addition, percent changes in [35S]methionine-labeled protein secretion from the PA-III cells incubated with 30 μg/ml bovine serum albumin (94 ± 6% of control values, n = 3) or conditioned media from 3T3 fibroblasts (101 ± 7% of control values, n = 3) and A431 cells (93 ± 8% of control values, n = 3) were not significantly different from the control cultures.

Stromal Cell-conditioned Media Stimulation of Polarized Protein Secretion from PA-III Cell Monolayers. Polarity of [35S]methionine-labeled protein secretion into the apical reservoir (Fig. 10, A, C, and E) and basal reservoir (Fig. 10, B, D, and F) under control conditions (Fig. 10, A and B) and when 30 μg/ml cold (nonlabeled) SCCM was added to the basal reservoir (Fig. 10, C and D) or the apical reservoir (Fig. 10, E and F) was investigated by two-dimensional polyacrylamide gel electrophoresis. In addition to SCCM stimulating a quantitative increase in [35S]methionine-labeled protein secretion (Fig. 9) from PA-III cells, SCCM (30 μg/ml) also induced the exclusive, basally directed secretion of a novel peptide, designated SE-1, from PA-III cells (Fig. 10, D and F) relative to control (non-stimulated) monolayers of PA-III cells (Fig. 10, A and B). This peptide (SE-1) has an isoelectric point of approximately 5.6, and an apparent molecular weight of approximately 95,000. This peptide was absent from the apical (Fig. 10A) and basal (Fig. 10B) secretions of control cultures, as well as from the apical secretions of SCCM-stimulated PA-III monolayers (Fig. 10, C and E). Furthermore, the stimulation of the basally directed secretion of peptide SE-1 occurred irrespective of whether the SCCM was added to the basal reservoir (Fig. 10, C and D) or the apical reservoir (Fig. 10, E and F) of the bicameral chambers. Pulse-chase studies of PA-III cell secretion into the basal reservoir indicated that 50% of the protein was secreted within the first hour (Fig. 12A) and could not be seen on two-dimensional gels at subsequent times of the chase period (not shown). In control cultures not stimulated by SCCM, peptide SE-1 was not secreted during the first hour (Fig. 12B) or at later times of the chase period (not shown).

**DISCUSSION**

After the growth of PA-III cells in bicameral chambers for 5 days the sheets of epithelial cells appeared morphologically...
paracrine stimulation of prostate secretion

Fig. 6. Prostatic cells isolated from the ventral prostate of 50-day-old rats and separated by isopyknic Percoll centrifugation. A and B, phase contrast image of stromal cells and the corresponding indirect immunofluorescence of vimentin intermediate filaments, respectively. C and D, phase contrast image of epithelial cells and the corresponding lack of vimentin immunofluorescence, respectively. Bars, 4.4 μm.

Fig. 7. Time course of [35S]methionine incorporation into PA-III cellular protein (cpm x 10^8/10^6 cells) in the absence (control) or presence of 30 μg/ml of SCCM placed in the basal reservoir of the bicameral chamber. Results are expressed as the means ± SE (bars) (n = 3).

Fig. 8. Time course of [35S]methionine incorporation into total PA-III cell secretory protein (cpm x 10^8/10^6 cells) in the absence (control) or presence of 30 μg/ml of SCCM placed in the basal reservoir of the bicameral chambers. Results are expressed as the means ± SE (bars) (n = 3).

Fig. 9. Dose-dependent influence of SCCM (μg/ml) on [35S]methionine-labeled protein secretion from PA-III cells. Protein secretion is expressed as a fold increase relative to control cultures in the absence of SCCM. Results are expressed as the means ± SE (bars) (n = 3).

confluent at the light (Fig. 2) and electron microscopic levels (Fig. 3, A and B). Concurrently, after the same period, the sheets of epithelial cells were characterized by a maximal electrical resistance. Electrical resistance has been widely used as an indicator of the formation of transepithelial permeability barriers (26) across cell lines of the canine kidney (27, 28), porcine kidney, toad urinary bladder (26), and human intestine (29) as well as in primary cultures of murine mammary epithelium (30) and rodent Sertoli cells (17). Since tight junctions are the rate-limiting permeability barrier of the paracellular channel across a confluent sheet of epithelial cells (31), the degree of electrical resistance has been correlated with the number of strands in the occluding junctions between adjacent cells (29). Indeed, differences in the electrical resistance of PA-III epithelial sheets grown in SFDM or FCS were paralleled by differences in the transepithelial flux of the extracellular space marker, [3H]inulin. Hence, based on the morphological observations, electrical resistance measurements, and [3H]inulin permeability studies, we conclude that PA-III cells grown to confluence in bicameral chambers and cultured, particularly in SFDM, prevent the mixing of fluids between the apical and basal reservoirs of the bicameral chambers. Indeed, these results are consistent with a compartmentalization of the milieu surrounding the epithelial monolayer into separate apical and basal domains, as normally occurs in vivo, thereby validating the prostatic bicameral chamber model for the examination of polarized epithelial cell secretion and paracrine interactions with underlying stroma or stromal cell-conditioned media.

The cytoplasm of vertebrate cells contain, in addition to microtubules and microfilaments, a third class of filamentous structures called intermediate filaments. Five major classes of intermediate filaments have hitherto been characterized both biochemically and immunologically (32). They are glial filaments found in glial cells; neurofilaments found in neurones; desmin filaments found predominantly in smooth, skeletal, and cardiac muscle cells; keratin filaments found in epithelial cells; and vimentin filaments found predominantly in mesenchymal cells (32, 33). The broad cross-reactivity of vimentin antibodies with mesenchymal cells of mammalian, avian, and amphibian
PARACRINE STIMULATION OF PROSTATE SECRETION

origin indicates that this protein is well conserved across the various phylogenetic classes (34–36). In this context, the primary antibody against murine vimentin used for indirect immunofluorescence readily cross-reacted with the rat prostatic stromal cells while not detecting any appreciable vimentin in the epithelial cells. Sherwood et al. (66) also found prominent expression of vimentin in prostatic stromal cells isolated from human benign prostatic hyperplasia tissue, with a very low level of expression in epithelial cells. Hence, we were able to use vimentin localization as a method of distinguishing between the rat stromal and epithelial cells. Following isopyknic Percoll centrifugation of the enriched stromal cell preparation of prostatic cells, we obtained a stromal cell fraction of 92% purity. This purity is comparable to that obtained by other workers (21, 37) who have used isopyknic sedimentation techniques to separate prostatic stroma from epithelial cells and have subsequently characterized the purity of their cell separation by the exclusive secretion of prostatic acid phosphatase by the epithelial cells. As a means of distinguishing between cell types within prostatic tissue, vimentin intermediate filament localization within stromal cells has several advantages over measuring epithelial prostatic acid phosphatase secretion. In this regard, vimentin is an extremely stable cytoskeletal component (38), whereas prostatic acid phosphatase is highly labile (39), requiring the addition of compounds such as citric acid to stabilize the enzyme. In addition, the expression of stromal vimentin is stable in vitro, even under serum starvation conditions (40), whereas the secretion of epithelial prostatic acid phosphatase

Fig. 10. Two-dimensional gel autoradiographs of [35S]methionine-labeled proteins secreted from the apical (A, C, E) and basal (B, D, F) surfaces of PA-III epithelial cells grown at confluence in bicameral chambers for 24 h. Control cultures (A, B) were grown in SFDM. Stromal cell conditioned media (30 µg/ml) added to the basal reservoir (C, D) or apical reservoir (E, F) of the bicameral chambers stimulated the exclusively basal secretion of the novel peptide SE-1 (D, F). Peptide SE-1 was absent from the corresponding apical secretions (C, E). Ordinates, molecular weight (x10^3); abscissas, isoelectric point. Autoradiographs are representative of 5 independent experiments, n = 5.

Fig. 11. Pulse-chase of [35S]methionine-labeled protein secretion (cpm x 10^4/10^6 cells) from the basal surface of PA-III cells stimulated by 30 ng/ml SCCM over a period of 12 h. Cultures were pulsed with 1.5 mCi/ml of [35S]methionine for 20 min and chased with a 1000-fold excess of [35S]methionine for up to 12 h. Secreted protein was collected at the indicated times. Results are representative of 3 experiments.

1971
secretion in a dose-dependent manner consistent with paracrine expression of Sertoli cell secretory proteins in a paracrine environment (48) where germ cells can induce the de novo example of ongoing growth and differentiation in the postnatal concept of stromal-epithelial interactions regulating fetal differentiation and development has also been extended to postnatal tissue remodeling where paracrine interactions between adjacent dissimilar cell types are considered to regulate localized growth. In this regard, the morphogenetic development of mammary tissue during pregnancy is considered to be a hormone-dependent epithelial-stromal regulated growth process (46, 47). Furthermore, the process of spermatogenesis is an example of ongoing growth and differentiation in the postnatal environment (48) where germ cells can induce the de novo expression of Sertoli cell secretory proteins in a paracrine regulatory manner (22). In this report we demonstrate that a trypsin-sensitive factor(s) with a molecular weight >10,000 in stromal cell-conditioned media stimulates PA-III epithelial cell secretion in a dose-dependent manner consistent with paracrine regulation of rat prostatic growth. Indeed, the preliminary results of Sherwood et al. (49) demonstrating stimulation of human prostatic epithelial cell growth by human stromal cell secretory proteins are comparable to the work reported herein. In addition, Swinnen et al. (67) recently demonstrated a factor in rat prostatic SCCM which could stimulate Sertoli cell secretion. Although several systemic growth factors such as insulin (50-52), epidermal growth factor (51), and prostatin (53, 54), as well as the autocrine prostate-derived growth factor (63, 64), are known to stimulate prostatic growth, the identity of the stromal cell-secreted paracrine protein(s) remains to be elucidated. Since live stromal cells were not required to stimulate PA-III epithelial cell secretion, the observed interaction was neither dependent on cell to cell contact nor due to the deposition of extracellular matrix by the stroma. Nevertheless, since the PA-III cells were seeded on Matrigel, the presence of a suitable substratum may be a permissive requirement for subsequent stromal stimulation of epithelium, as has been demonstrated for mammary tissue (55). Indeed, the observations that stromal stimulation of mammary epithelial cell secretion may be exerted from multiple stromal cell types (56) present the possibility that the diverse population of stromal cells within the prostate may secrete different stimulatory factors and that this diverse population of stromal cells may differentially regulate epithelial secretion and growth during development and in the postnatal environment.

Many differentiated cells are morphologically polarized and express secretory proteins in a vectorial manner consistent with a domain-specific interaction with the surrounding milieu and/or adjacent cells. In vitro, vectorial secretion by Madin-Darby canine kidney cells (57, 58), mammary epithelium (59), and Sertoli cells (22, 60) is considered to reflect compartmentalization of tissue function of the in vivo counterparts of these cells (18). In the rat prostate our work shows that the stromal cell protein(s) induced the vectorial secretion of a novel peptide (SE-1) exclusively from the basal surface of the neoplastic PA-III epithelial cells. Curiously, the induction of peptide SE-1 secretion occurred irrespective of whether the stromal cell proteins were added to the apical or basal surface of the PA-III cells. This could be due to the missorting and aberrant expression of corresponding receptors on the apical plasmalemma of the transformed PA-III cells. Alternatively, the stromal cell proteins may have been endocytosed from the apical surface of the PA-III cells, nonspecifically (fluid phase), and following internalization stimulated peptide SE-1 expression. Even though the stimulation of peptide SE-1 secretion was not domain specific, the secretion of peptide SE-1 was domain specific. Hence, the stromal cell protein induction of peptide SE-1 secretion from the basal domain of the PA-III epithelial cells in the direction where stromal cells would normally reside in vivo is consistent with a compartmentalized paracrine interaction between prostatic epithelia and stroma in the regulation of prostatic secretion. In this context, it would be of interest to determine whether the stromal cell protein induction of peptide SE-1 secretion from epithelial cells normally occurs in vivo, in which case the peptide could be used as a marker of paracrine regulated growth. Considering the variable and often unpredictable fluctuations in the secretion of prostate-specific antigen and prostatic acid phosphatase used as serum markers of malignant carcinomas (61, 62), measurement of an epithelial protein such as peptide SE-1 which is basally secreted, and therefore may occur in serum, may prove to be a more reliable and specific marker for the early diagnosis of malignant prostatic carcinomas.

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