Biochemical Analysis of Secretory Proteins Synthesized by Normal Rat Pancreas and by Pancreatic Acinar Tumor Cells

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ABSTRACT   We have examined the secretogogue responsiveness and the pattern of secretory proteins produced by a transplantable rat pancreatic acinar cell tumor. Dispersed tumor cells were found to discharge secretory proteins in vitro when incubated with hormones that act on four different classes of receptors: carbamylcholine, caerulein, secretin-vasoactive intestinal peptide, and bombesin. With all hormones tested, maximal discharge from tumor cells was only about one-half that of control pancreatic lobules, but occurred at the same dose optima except for secretin, whose dose optimum was 10-fold higher. Biochemical analysis of secretory proteins discharged by the tumor cells was carried out by crossed immunoelectrophoresis and by two-dimensional isoelectric focusing-SDS polyacrylamide gel electrophoresis. To establish a baseline for comparison, secretory proteins from normal rat pancreas were identified according to enzymatic activity and correlated with migration position on two-dimensional gels.

Our results indicate that a group of basic polypeptides including proelastase, basic trypsinogen, basic chymotrypsinogen, and ribonuclease, two out of three forms of procarboxypeptidase B, and the major lipase species were greatly reduced or absent in tumor cell secretion. In contrast, the amount of acidic chymotrypsinogen was notably increased compared with normal acinar cells. Although the acinar tumor cells are highly differentiated cytologically and express functional receptors for several classes of pancreatic secretagogues, they show quantitative and qualitative differences when compared with normal pancreas with regard to their production of secretory proteins.

The acinar cell of the mammalian pancreas is highly differentiated and adapted for the synthesis, packaging, and discharge of secretory proteins. The anatomic organization of organelles within the cell expedites the secretory process in that the protein synthetic apparatus is located in the basal pole of the cell while secretory granules accumulate in the apical pole. Under physiologic conditions, hormones interact with receptors located on the basal plasmalemma, which in turn leads to granule release at the apical membrane. Specialized regions of the plasma membrane may play a role in directing vectorial release of secretory proteins. Evidence for such specialization includes differences in intramembrane particle distribution between basolateral and apical membranes as shown by freeze-fracture electron microscopy (1) as well as by marked differences in distribution of lectin binding sites (2). As we have shown in previous papers (3, 4), rat pancreatic acinar tumor cells lack polarized organization of the plasmalemma as deter-

mined by either freeze-fracture techniques or lectin binding. Though the acinar tumor cells contain the cellular machinery for the production and packaging of secretory proteins, it is not known whether the lack of polar organization of the plasmalemma compromises their response to peptide secretagogues.

In this investigation we sought to determine whether rat acinar tumor cells are capable of responding to hormones representative of four known classes of acinar cell secretagogues (5) and whether or not secretory proteins synthesized and discharged from the tumor cells differ from those of the normal pancreas. Our results show that the acinar tumor cells discharge secretory proteins in response to several secretagogues, extending the report by Warren and Reddy (6) that acinar tumor cells respond to carbachol and that qualitative and quantitative differences can be detected among the tumor cell secretory proteins when compared with those of the normal

rat pancreas. Portions of this work have appeared in abstract form (7).

MATERIALS AND METHODS

Materials

Chemicals were obtained from the following sources: enterokinase, phenylmethylsulfonyl fluoride (PMSF), and synthetic enzyme substrates, Sigma Chemical Co., St. Louis, MO; trypsin (three times crystallized) and soybean trypsin inhibitor (SBTI), Worthington Biochemical Corp., Freehold, NJ; bovine serum albumin, Armour Pharmaceuticals, Phoenix, AZ; [^{35}S]methionine, Amersham, Corp., Arlington Heights, IL; Trasylol, FBA Pharmaceuticals, New York, NY. Hormones were obtained from the following suppliers: caerulein, Pharmitalia, Milan, Italy; synthetic secretin, Squibb Institute for Biomedical Research, Princeton, NJ; vasoactive intestinal peptide (VIP), courtesy of Dr. V. Mutt, GIH Research Laboratories, Karolinska Institute, Stockholm, Sweden; bombesin, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, CA.

Tumor Transplantation

Tumor transplantation was carried out in Sprague-Dawley Fischer 344 rats as described in the preceding paper (3). Tumors were taken from passages 14–20 and were grown subcutaneously, intraperitoneally, or intrapancreatically. Tumors taken for experiments ranged from 2 to 5 cm in diameter and were derived from subcutaneous growths except where indicated.

Tumor Dissociation

Animals were killed by decapitation and tumors were placed into Krebs-Ringer-Hepes (KRH) medium (8). The tumor capsule was cut open and the soft tissue was scraped out with a spatula. Tissue clumps were minced with scissors and washed in medium by decantation until cleared of red blood cells. The tissue was mixed 1:1 with the same medium and passed five times through a 10-ml Kimax pipette (1.2-mm tip diameter). Large fragments of connective tissue and blood vessels were removed by filtration through a 1-mm nylon mesh. The filtrate was then further mechanically dispersed by three passes through a Pasteur pipette (flamed to a 0.5 mm tip diameter). Finally, the cells were filtered through 0.2-mm nylon mesh and washed three times by centrifugation (2 min at 500 g). Mechanical treatment of the tissue was sufficient to achieve dissociation, probably due to lack of basal lamina and incomplete tight junctions in the acinar tumor as we described in the previous paper (3).[1] All glassware used for tissue dissociation was siliconized before use.

Assay for Secretory Protein Discharge

Mechanically dissociated tumor cells or pancreatic lobules (9) prepared from normal Fischer 344 rats were pulse-labeled for 10 min with [^{3}H]leucine (5 μCi/ml) in KRH at 37°C, washed twice with KRH containing an excess of unlabeled leucine (4 mM final concentration), and resuspended in the same medium. After addition of secretagogues, cells or lobules were incubated for 2 h at 37°C with constant agitation under 100% O$_2$. Tissue and incubation media were separated by centrifugation (500 g for 8 min). The tissue was then placed in 1 ml of water and dispersed by sonication. One-tenth ml of 1% BSA was added to the sonicates and to the collected medium, which were then precipitated with TCA at 4°C. TCA precipitates from tissue and medium were washed two times with cold 5% TCA by centrifugation. Pellets were dissolved in formic acid before liquid scintillation counting as described previously (10). Percent secretion was defined as radioactivity present in proteins from the medium divided by total radioactivity in tissue plus medium times 100.

Labeling of Secretory Proteins of Rat Pancreatic Lobules and Dissociated Tumor Cells

Dissected lobules or dissociated tumor cells were placed in KRH containing 100 U/ml Trasylol and 10 μg/ml SBTI without BSA. The total volume was 3–5 ml and contained 0.3–0.5 ml of settled cells or lobules. [^{35}S]methionine was added at 50 μCi per ml and the tissue was incubated for 3 h at 37°C with continuous shaking in the presence of 10^{-8} M caerulein. The incubation medium containing discharged secretory proteins was centrifuged at 500 g for 2 min to remove lobules

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[1] Application of a collagenase digestion procedure similar to that used to obtain acini from the normal pancreas (8) was less satisfactory than mechanical dissociation, since it produced more cell damage as manifested by poor response to secretagogues.

or cells, and the supernatant was subjected to high-speed centrifugation (100,000 g$_{avg}$ for 30 min) to remove cellular debris. The supernatant was aliquoted into tubes and rapidly frozen in liquid N$_2$.

Measurement of Actual and Potential Enzyme Activities Separated by Isoelectric Focusing

Secretion obtained from normal rat pancreas and from acinar tumor cells was diluted 1:10 to a final concentration of 50–100 μg/ml with a buffer containing 0.1 M Tris HCl, pH 8.0, 0.02 M CaCl$_2$, 0.1 M KCl, and 100 μg/ml BSA. The activation conditions were as follows: trypsinogen was activated at room temperature for 30 min with 0.01 μg/ml enterokinase; chymotrypsinogen was activated at 4°C with 1 μg/ml trypsin for 2 h; procarboxypeptidase A and B were activated with 1 μg/ml trypsin for 1–2 h at room temperature; and proelastase was activated with 2 μg/ml trypsin at 4°C for 16 h. Preparative isoelectric focusing (IEF) of secretory proteins and subsequent elution of proteins from the gel slices was carried out as described by Scheele (11). Zymogens eluted from IEF preparative gels were activated by the same procedure.

Enzymatic activities were measured as follows: amylase was determined according to Bernfeld (12); lipase was measured using a nephelometric procedure with olive oil emulsion (13); ribonuclease was determined according to Kalnitsky et al. (14); tryptic activity was measured using TAME-HCl as substrate (15); and chymotryptic activity was measured with N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate (16). Procarboxypeptidase A and B activities were assessed using hippuryl phenylalanine and hippuryl arginine, respectively (17). Elastase activity was determined using insoluble ^{125}I-elastin as substrate according to the method of Robert and Robert (18).

Two-dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis was carried out as described by Scheele (11) with the following modifications. Trasylol (20 U/ml) and PMSF (1 mM) were included in the IEF gels, and 1 mM PMSF was present in the SDS stacking gels (19). Without these additions, extensive proteolytic degradation of secretory proteins was noted. Gels were stained with 0.25% Coomassie Blue in 30% methanol/7% acetic acid and destained with 30% methanol/7% acetic acid. Fluorography was performed by the procedure of Bonner and Laskey (20) on [^{35}S]methionine-labeled proteins that were separated by two-dimensional gel electrophoresis. Films were exposed for 5–14 d at −70°C.

Immunological Techniques

Rabbit antibodies against normal Fischer 344 rat pancreatic secretory proteins were prepared as described in the preceding paper (3). Crossed-immunoelectrophoresis was performed with 1% agarose in 100 mM veronal acetate buffer (pH 8.6) used as a matrix on 4 × 4 cm glass slides. The first dimension was run at 15 mA for 2 h at 4°C with 10–20 μg of protein in the load. The separated proteins were run in the second dimension into a new gel containing 1–2 mg of purified antisecretory protein IgG per ml of agarose. Electrophoresis was performed at 170 V for 16–18 h at 4°C. 1 mM PMSF was included throughout to inhibit proteolysis. Gels were washed extensively with 0.9% NaCl and with distilled water for 2 d, dried, and stained with 0.05% Coomassie Blue in 30% methanol/7% acetic acid.

Other Techniques

Microscopy of dispersed tumor cells was carried out as described in the preceding paper (3). Proteins were measured by the procedure of Lowry et al. (21).

RESULTS

Morphological Appearance of Dissociated Tumor Cells

Mechanical dissociation of the acinar tumor yielded clumps of cells (from 4 to 10) with a viability of 90–95% as shown by exclusion of trypan blue (Fig. 1) and linear incorporation of [^{3}H]leucine into total cell proteins for up to 4 h (data not shown). Thick and thin sections of plastic-embedded samples also showed small clumps of cells and single tumor cells whose overall morphologic preservation was good except for occasional cytoplasmic vacuoles (Fig. 2).
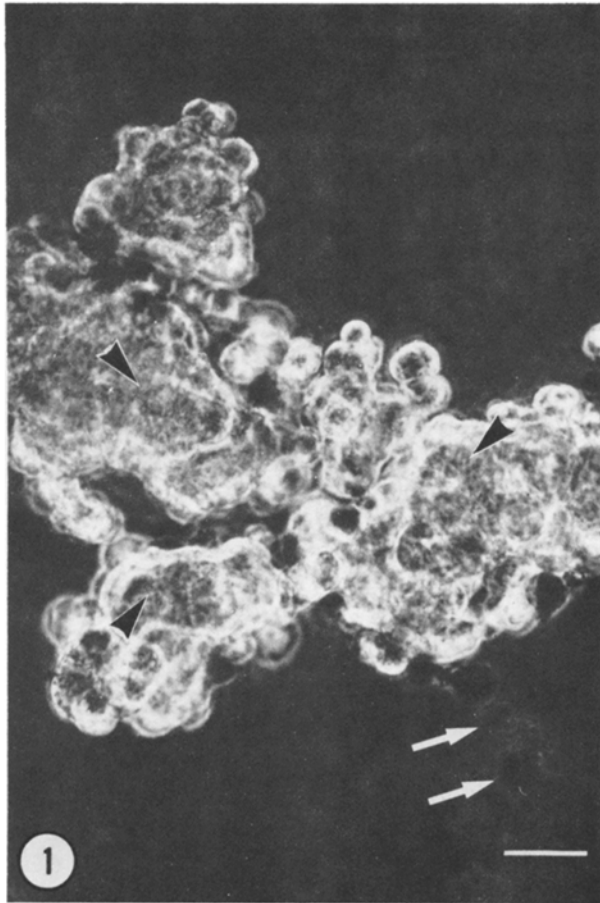
FIGURE 1 Light micrograph of freshly dispersed acinar tumor cells exposed to 1% trypan blue and viewed by phase-contrast microscopy. Note that only a few cells at the periphery of the tumor cell clumps take up trypan blue (arrows). Arrowheads indicate fields of secretory granules. Bar, 20 μm. × 550.
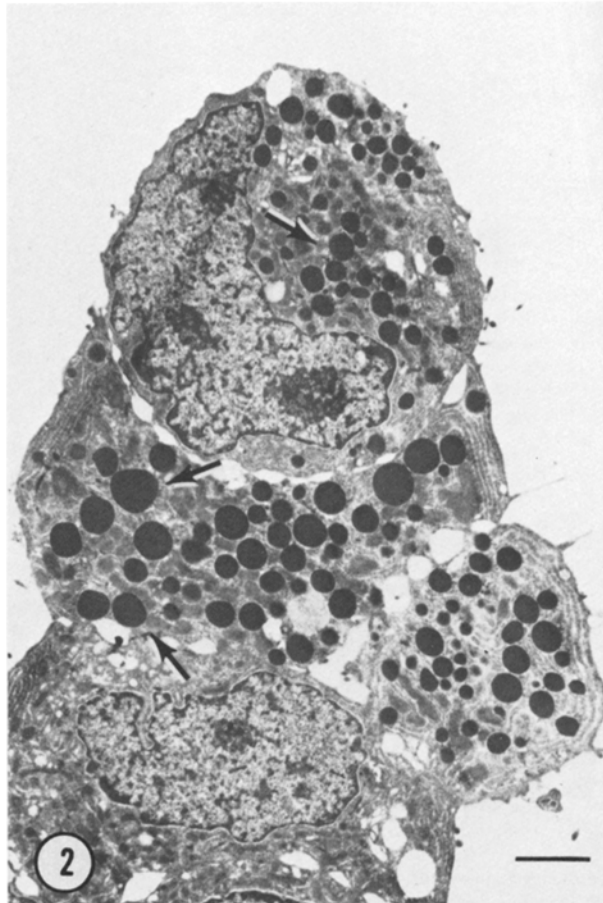
FIGURE 2 Low-power electron micrograph of a mechanically dispersed acinar tumor cell preparation similar to that shown in Fig. 1. Note that the cells assume a variety of shapes in the preparation. Arrows indicate secretory granules. The cell at the top of the field contains secretory granules in a supranuclear zone and is similar morphologically to dispersed single cells of normal pancreas (22). Bar, 2 μm. × 5,000.

Response to Secretagogues

Biosynthetic labeling of secretory proteins by a pulse-chase protocol previously described (23) provided a simple assay for examination of the response of pancreatic cells to a variety of secretagogues. Because tumor cells were mechanically dissociated, we used as controls lobules dissected from Fischer 344 rat pancreas instead of enzymatically dispersed acini. The tumor cells responded to five secretagogues (Table I), which interact with four different receptor systems: carbachol with muscarinic cholinergic receptors, caerulein with cholecystokinin receptors, VIP and secretin with secretin receptors, and bombesin with its separate receptor (5).

During a 2-h incubation period, maximum discharge of twofold over basal levels was elicited in tumor cells by each secretagogue studied. In contrast, three- to fourfold stimulation over basal levels was found in normal rat pancreatic lobules. The concentration of secretagogues needed for maximum release from tumor cells and from normal pancreatic lobules was similar in the case of caerulein, carbachol, VIP, and bombesin. However, for secretin the concentration of hormone required was 10-fold higher than that needed for normal tissue.

TABLE I

Response of Acinar Tumor Cells and of Normal Pancreatic Lobules to Secretagogues

Secretagogue	Concentration (M)	% Secretion (± 1 SD)	
		Tumor	Lobules
None	—	13.6 ± 2.1 (10)	9.0 ± 2.1 (9)
Carbachol	10^{-5}	20.4 ± 1.8 (7)	27.7 ± 4.7 (8)
Caerulein	10^{-9}	21.6 ± 1.9 (6)	31.5 ± 2.8 (6)
Bombesin	10^{-6}	20.2 ± 1.9 (5)	30.1 ± 4.7 (3)
Secretin	10^{-7}	20.6 ± 0.8 (2)	15.8 ± 0.6 (2)
VIP	10^{-8}	22.1 ± 6.1 (2)	19.4 ± 3.5 (2)

Secretory proteins were biosynthetically labeled with [^{3}H]leucine, and percentage discharge of labeled proteins was assayed as described under Materials and Methods. Numbers in parentheses indicate number of individual experiments performed.

Immunological Analyses of Secretory Proteins

We compared the secretory proteins from the acinar cell tumor and from normal pancreatic tissue by immunological
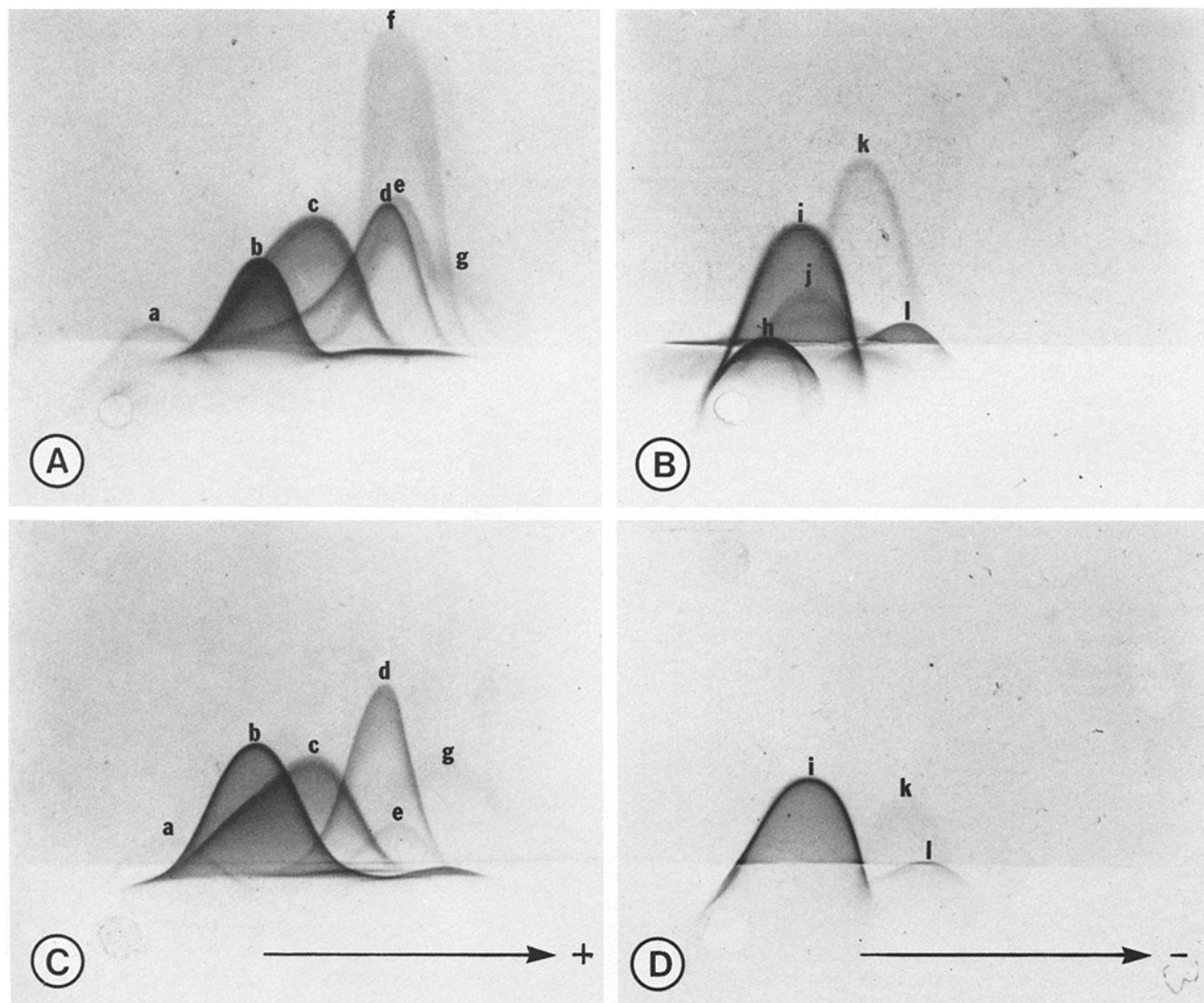
FIGURE 3 Crossed immunoelectrophoresis of normal (A, B) and tumor (C, D) secretory proteins. In A and C the secretory proteins were run toward the anode and in B and D they were run toward the cathode. In the second dimension, the secretory proteins were electrophoresed into an agarose gel containing rabbit anti-rat secretory protein antibodies.

and biochemical techniques. Antibodies against the secretory proteins of Fischer 344 rat pancreas (tested by double diffusion in agarose plates) precipitated proteins obtained from discharged secretion as well as from homogenates of the tumor and normal pancreas. Indirect immunofluorescence, as noted in the previous paper (3), also showed that these antibodies stain secretory granules selectively in both normal and acinar tumor cells.

We carried out crossed-immunoelectrophoresis of both normal and tumor secretion using independent analyses of the acidic and basic proteins to provide optimal resolution of immunoprecipitation patterns. For this purpose, aliquots of normal and tumor secretion, identical with regard to both protein concentration and volume, were electrophoresed in parallel toward the positive and negative electrodes.

The acidic proteins from normal rat pancreatic secretion were resolved into seven major peaks and the basic proteins into five peaks. Overall, crossed immunoelectrophoresis resolved 12 major antigens (Fig. 3). Analysis of the tumor secretion revealed major differences in the pattern of the basic

polypeptides when compared with control secretion in that only three out of five antigens were detected; two of the precipitin rockets (k and l in Fig. 3) were reduced by 90% as estimated by area under the peak. The antigen identified as amylase[2] (i in Fig. 3 B and D) was not significantly decreased in the tumor. We also observed that the acidic polypeptides of tumor secretion were missing antigen f (Fig. 3 C). Other changes in the relative proportions of several antigens based on inspection of areas were noted (compare Fig. 3 A and B to C and D).

The immunoelectrophoretic analysis thus showed that tumor secretory proteins differed qualitatively and quantitatively from those of normal rat pancreatic secretion. However, because these techniques may not detect immunologically modified tumor secretory proteins that are absent in normal secretory proteins used as immunogen, we sought to further char-

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[2] The identification was carried out by precipitation with shell fish glycogen, which selectively removes amylase (24).

acterize acinar tumor secretory proteins by two-dimensional gel electrophoresis.

Characterization of Discharged Secretory Proteins from Normal Rat Pancreas

Although two-dimensional gel electrophoresis has been utilized to identify several of the polypeptides of adult rat pancreatic secretion (25, 26), a detailed analysis of their properties has yet to be performed. Such an analysis was necessary to provide a baseline for comparison with secretory proteins produced by the rat acinar cell tumor. To this end, we have used two-dimensional gel electrophoretic analysis as described by Scheele for guinea pig pancreatic secretion (11). Secretory proteins hormonally discharged by rat pancreatic lobules were first applied to IEF gels. For separation in the second dimension, strips of IEF gels were mounted on the top of 10–20% SDS-polyacrylamide gel gradients. Analysis of the two-dimensional gel showed that 17 major Coomassie Blue-stained spots were detectable in normal pancreatic secretion (Fig. 4A). All spots detected by Coomassie Blue staining (except for exogenous SBTI) were radiolabeled following incorporation of [^{35}S]methionine as revealed by fluorography of dried gels (Fig. 4 B). As was noted by Tartakoff et al. (27), high rates of amino acid incorporation are distinctive properties of secretory proteins synthesized by the pancreas.

To identify these polypeptides, we assayed the enzymatic activities of secretory proteins eluted from strips of preparative IEF gels. Activation was necessary to detect all proteases tested which confirms that these enzymes were present as zymogens. The enzymatic activities found in the IEF gel are summarized in Fig. 5. Several enzymatic activities were present in multiple forms. Thus, we found three forms of trypsinogen, an acidic form (pI 4.2, spot no. 2 on the two-dimensional map) and two basic forms (pI 7.6, spot no. 3; pI 8.2, spot no. 4). Only two species have been previously reported (28). Chymotrypsinogen was also shown to have an acidic form (pI 4.4, spot no. 5) and a basic form (pI 8.2, spot no. 6) confirming the observations of Pascale et al. (29). It has been previously reported for pancreatic secretion from guinea pigs (11) and humans (19) that acidic chymotrypsinogen forms a complex with procarboxypeptidase B. In the case of rat secretory proteins, the activity of procartrypsinogen. In the two-dimensional gel, procarboxypeptidase B activity coincided with three separate spots numbered 8–10 with pI's of ~4.4. However, we do not know whether all three spots represent this enzyme activity. Procarboxypeptidase A activity could not be detected under the conditions employed. The minor spots 12 and 13 on the two-dimensional map remain unidentified. Proelastase was localized at the basic edge of the IEF gel with a pI of 8.2 (spot 7) and focused near basic chymotrypsinogen.

Amylase was present in two forms differing in isoelectric point on IEF gels and usually appeared as an apparent pair of doublets in SDS gels (spots 14–17). Lipase activity was present in a minor acidic form (pI 4.3) and as a major acidic species (spot 11, pI 6.0). Two species of rat pancreatic lipases have also been described previously (30). The molecular weight of the minor acid lipase has not been determined; it may correspond to the high molecular weight protein present above spot 10. The identification of rat secretory proteins within two-dimensional gel electrophoretic maps, summarized in Table II, makes possible a study of the altered secretory proteins of the rat acinar cell tumor.

Tumor Secretory Proteins

We next examined the composition of secretory proteins synthesized by the acinar tumor cells. Dissociated acinar tumor cells, labeled with [^{35}S]methionine, were stimulated to secrete by treatment with 10^{-8} M caerulein, and the secretory proteins discharged into the medium were separated by IEF-SDS PAGE. The pattern of the tumor secretory proteins (Fig. 4) showed the following differences when compared to normal rat pancreatic secretory proteins. (a) Basic secretory proteins such as proelastase, basic chymotrypsinogen, basic trypsinogen, and RNase were either absent or barely detectable by either Coomassie Blue staining or fluorography (Fig. 4 C and D). (b) The major form of lipase was not detected nor were two out of three forms of procarboxypeptidase B (spots 8 and 9). (c) Spots 12 and 13, which remain unidentified enzymatically, were also markedly reduced or absent. These observations are in agreement with our immunological results presented above.

It is well established that nutritional factors may induce changes in the composition of pancreatic secretory proteins (31, 32). Location of the pancreatic tumor outside of the gastrointestinal tract may therefore have caused the changes observed in tumor secretory proteins. To test this possibility, we analyzed secretory proteins discharged from cells of tumors growing directly in the pancreas and in the peritoneal cavity. As the rat acinar cell tumor is separated from surrounding tissue by a thick connective tissue capsule, contamination of the tumor material by adjacent normal pancreas is avoided. We found that the pattern of tumor secretory proteins was identical regardless of the site of tumor growth, suggesting that variations in nutrients dependent on the vascular supply likely are not responsible for the differences between tumor and normal pancreatic secretion.

DISCUSSION

Our studies on the morphological features of a rat pancreatic acinar cell tumor have shown that whereas secretory granules usually are located in a defined zone of the cytoplasm, no obvious apical and basolateral specialization of the plasmalemma is noted in cells of the tumor parenchyma (3). Despite the differences in morphology of tumor cells and normal acinar cells, as reported here tumor cells are able to discharge secretory proteins in vitro in response to hormonal stimuli. Secretion assays show that the acinar tumor cells respond to a variety of secretagogues including carbachol, caerulein, secretin, vasoactive intestinal peptide, and bombesin which are representative of ligands for major known classes of secretagogue receptors in the acinar cell (5). From these observations we conclude that the tumor cells possess a functional set of receptors similar to those on the surface of normal pancreatic cells. Our observations confirm and extend the work of Warren and Reddy (6) who previously reported that the acinar tumor cells are capable of responding to carbachol.

Though tumor cells possess functional secretagogue receptors, the maximal induced discharge was twofold lower than that from normal pancreatic lobules. This decreased hormone sensitivity may reflect alterations of the cell surface such as changes in receptor number or affinity, inefficient fusion of the plasma membrane with the membrane of zymogen granules, or fewer numbers of granules per tumor cell. Further investigation is required to clarify such potential differences between normal and neoplastic acinar cells.

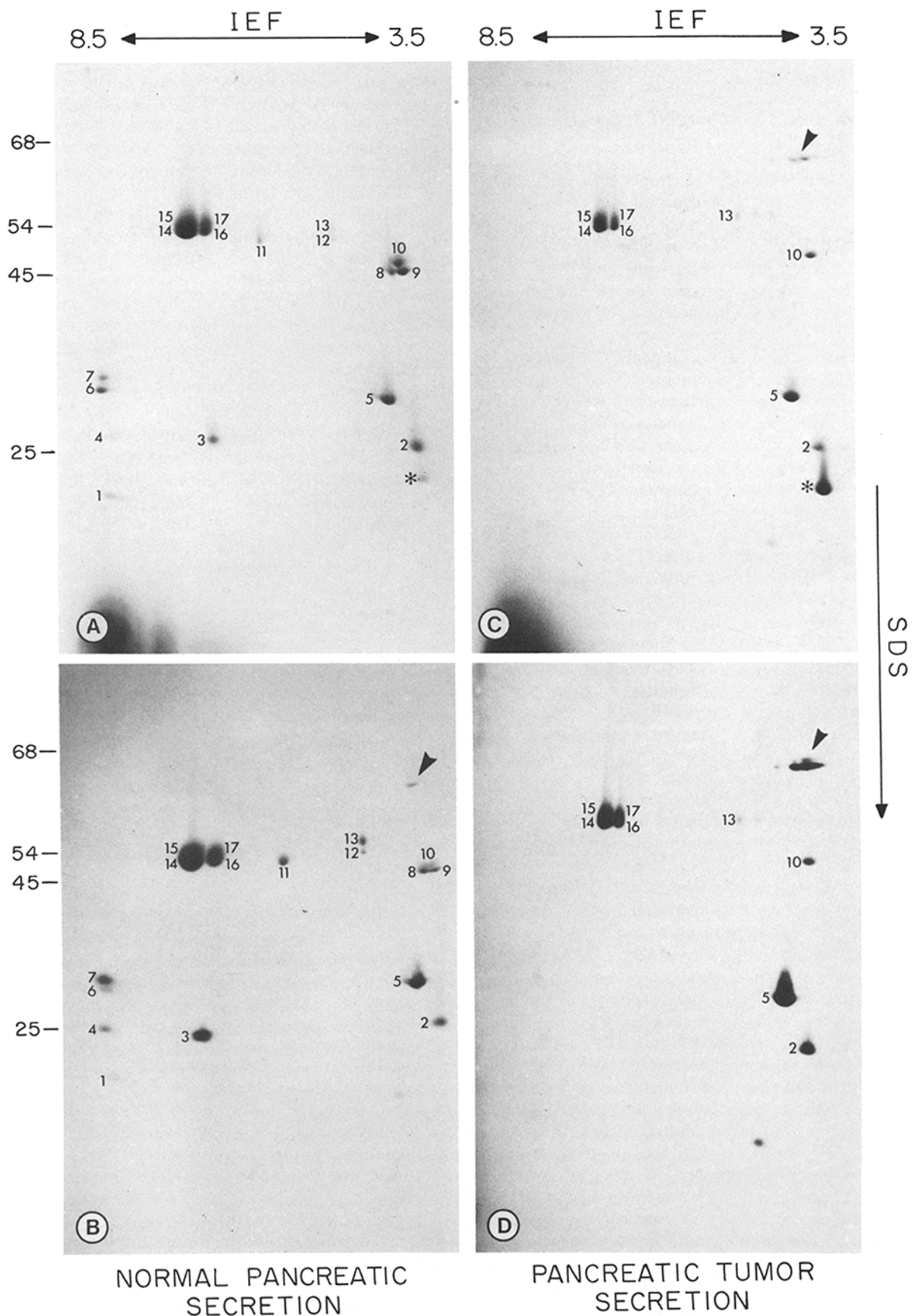As indicated by the studies of Reddy et al. (33), several

FIGURE 4   Two-dimensional IEF-SDS gel electrophoresis of normal pancreatic (A, B) and tumor (C, D) secretory proteins. A and C are the Coomassie-Blue-stained patterns of secretory proteins. The asterisk indicates the position of SBTI added to the incubation medium. B and D are the corresponding fluorograms of ^{35}S-methionine, biosynthetically labeled proteins. The arrowheads indicate the possible position of the minor acidic form of lipase. The numbers 1–17 on the gels mark positions of the secretory proteins whose enzymatic identification is given in Table II. Numbers on the left indicate $M_r \times 10^{-3}$ determined from molecular weight standards: BSA, 68×10^3; porcine amylase, 54×10^3; ovalbumin, 44.8×10^3; bovine trypsinogen, 25×10^3. Representative of 10 separate experiments.
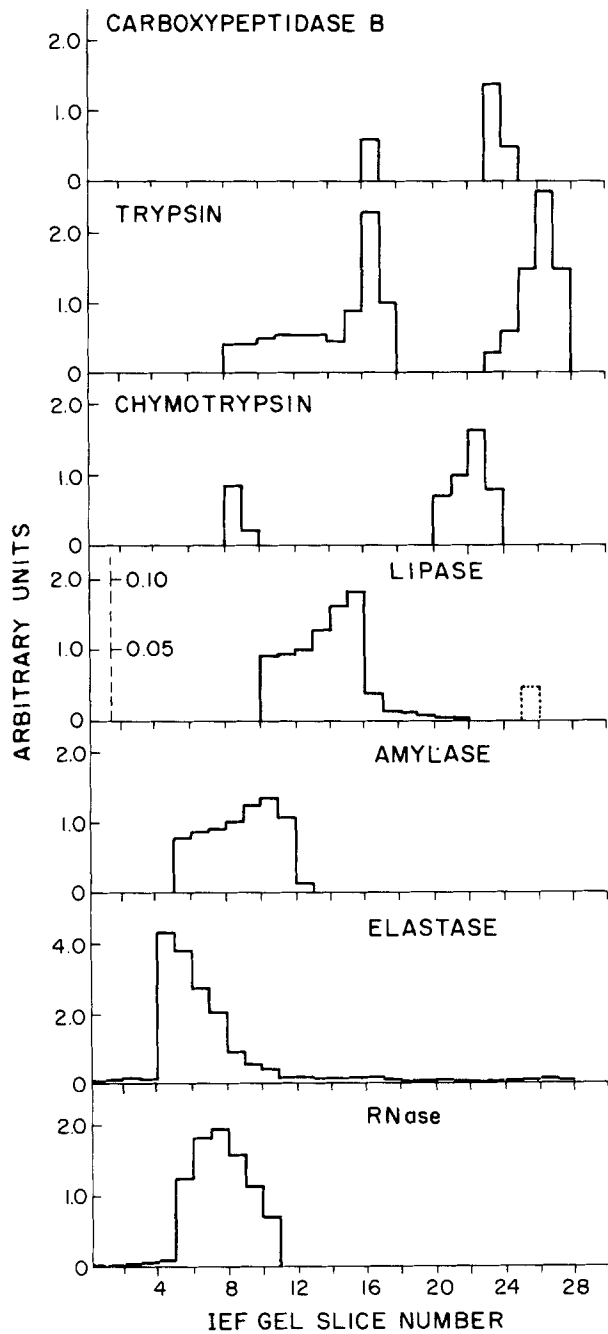
enzyme activities present in normal rat pancreas are found in secretory granules isolated from rat acinar tumor cells. As reported in the present study, further analysis of secretory proteins both by crossed immunoelectrophoresis and by two-dimensional gel electrophoresis shows that there are marked qualitative and quantitative differences in the patterns of secretory proteins from acinar tumor cells compared to those from normal acinar cells. As summarized in Table II, the major lipase species and two out of three spots corresponding with procarboxypeptidase B activity were not detected; RNase, basic chymotrypsinogen, basic trypsinogen, and proelastase are greatly decreased and detected only by extended autoradiography; and the amount of acidic chymotrypsinogen is increased in the tumor cells as evidenced by fluorography of two-dimensional IEF-SDS gels.

Although several reports (34, 35) indicate that differences may exist in the types of secretory proteins collected from the pancreatic juice of patients with pancreatic carcinoma, to our knowledge it is not clear whether a correlation exists between the histology of the tumor and the secretory protein profile. In view of the fact that up to 13% of human pancreatic cancers may be of acinar cell origin (36), it will be of interest to compare the pattern of secretory proteins of these patients with that from normal subjects as well as to correlate secretory protein patterns with the histology of pancreatic neoplasms in general.

The differenes noted here in the secretory protein pattern from acinar tumor cells may reflect a unique phenotypic expression of this neoplasm. Alternatively, they may represent a pattern of secretory protein production that mimics that of normal developing rat pancreas (4). For this reason, as described in the following paper, we compared the secretory protein patterns found in the tumor cells with those of normal developing rat pancreatic rudiments.

FIGURE 5   Position of enzymatic activities eluted from IEF gel slices. The gel was cut into 0.5-cm slices before extraction. The left- and right-hand sides of the histogram represent the basic and acidic ends of the gel, respectively. Enzymatic activities are expressed in arbitrary units based on standard enzyme assays, except for elastase activity, which is given as TCA-soluble cpm $\times$ 10^{-3} released/h from ^{125}I-labeled elastin. Representative of at least four separate experiments. Note that, in the case of lipase, two scales are given due to differences in the activities of the lipase species.

TABLE II

Correlation of Rat Pancreatic Secretory Protein Map With Enzyme and Proenzyme Activities

Peptide no.	Enzyme or proenzyme	pI	M$_r$	Presence in tumor secretion
1	RNase	8.2	14,000	−
2	Acidic trypsinogen	4.2	25,500	+
3	Basic trypsinogen	7.6	26,000	−
4	Basic trypsinogen	8.2	26,500	−
5	Acidic chymotrypsinogen	4.4	28,500	++
6	Basic chymotrypsinogen	8.2	30,000	−
7	Proelastase	8.2	32,000	−
8–10	Procarboxypeptidase B	4.4	46,000–49,000	−(8, 9) + (10)
11	Lipase	6.0	49,000	−
12, 13	Unidentified	5.6	49,000–52,000	±
14–17	Amylase complex	7.5–8.0	50,000–54,000	+

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