Characterization of Somatostatin Specific Binding in Plasma Cell Membranes of Human Placenta

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Summary

Somatostatin is a known inhibitor of hormone secretion and of nutrient transport. Because somatostatin-like immunoreactivity has been detected in amniotic fluid and the placenta has both hormone secretory and nutrient transport functions, we investigated the possible existence of somatostatin receptors on placenta cell membranes. Binding of ^{125}I-Tyr^1- and ^{125}I-Tyr^{11}-somatostatin (5–21%) to solubilized placenta cell membranes was observed. Binding was time-, temperature-, and pH-dependent and occurred maximally with incubation at concentrations of 25 μg of membrane protein. Displacement of binding of ^{125}I-Tyr^1 and Tyr^{11} somatostatin by cold cyclic and linear somatostatin and somatostatin analogs Ala-5, Ala-8, and Ala-11 was observed. Scatchard analysis of data revealed high capacity of (R_o 0.44 mol/μg × 10^{-12}) but low affinity (K_d 1.8 M × 10^{-7}) binding sites similar to that reported in other tissues. Binding was not reversible under our experimental conditions. The significance of this low affinity binding of somatostatin to placenta cell membranes remains to be determined.

Abbreviation

SRIF, somatostatin

Somatostatin has been shown to have widespread inhibitory actions on the secretion of several hormones (8) and on nutrient transport across the gut (12, 23). The placenta is important for both hormone secretion and nutrient transport. The role of somatostatin in the regulation of placental hormone secretion or nutrient transport has not been investigated extensively (17). The abundance of somatostatin in the amniotic fluid (6) and its presence in placental tissue demonstrated by immunohistochemical and extraction techniques (5, 13, 19, 26) suggests a possible role of this hormone on placental function. Because most of the evidence suggests that somatostatin acts on target cells via a membrane receptor site (15), we examined placenta cell membranes for the presence of specific somatostatin receptors. Human placental tissue is readily available and has been previously used to study binding kinetics of polypeptide hormones such as insulin and some of the somatomedins (10, 18).

Somatostatin-binding sites have recently been demonstrated in the cell membranes of some of its presumed target tissues, such as the pituitary gland (14), brain neural synaptosomes (14), and pancreatic islet secretory vesicles (25). In addition somatostatin binding to intact cells of the gastric mucosa (20), adipocytes (15), and pituitary cells in culture have been reported (21). In

the latter, the presence or absence of binding sites correlated with the ability of somatostatin to inhibit growth hormone and prolactin release, thus suggesting that the action of somatostatin may be mediated through binding sites on the cells.

MATERIALS AND METHODS

Linear and cyclic somatostatins were purchased from Bachem Inc., Marina del Rey, CA. Somatostatin analogs (Ala-5, Ala-8, and Ala-11) were kindly provided by Dr. J. E. Rivier of the Salk Institute. Tyr^1-somatostatin and Tyr^{11}-somatostatin were purchased from Calbiochem. Other chemicals used were Triton X-100 for solubilization of the placenta cell membranes, Bio-Beads SM-2 (Bio-Rad, Inc.) for the removal of the Triton X-100, toluene sulfonyl fluoride (Eastman), charcoal (Fisher), and dextran T-70 (Sigma).

Preparation of membranes. Human placenta from five normal term pregnancies, one 32-week, and one diabetic pregnancy were obtained 10–15 min after delivery and transported on ice. All further procedures were performed at 4°C. Cell membranes were prepared from all placentas and tested for total and nonspecific binding of somatostatin.

Placental tissue, both fetal and maternal, was minced with scissors and rinsed in cold (0.3 M) sucrose. The tissue was placed in a volume of 0.3 M sucrose equal to 1.5 × tissue volume and homogenized using a Polytron homogenizer. The homogenate was then centrifuged for 10 min at 600 × g; the supernatant was recentrifuged at 12,000 × g for 30 min and this supernatant was saved. To each 100 ml of supernatant, 0.58 g of NaCl (0.1 M) and 4.92 mg MgSO$_4$ (0.2 mM) were added. This solution was centrifuged at 45,000 × g for 45 min; the pellet was washed and resuspended three times in 0.1 M NaPO$_4$ buffer (pH 7.4). Final pellets were suspended in 10 ml of buffer and gently homogenized in a hand-held glass homogenizer.

The above preparation constituted the particulate cell membranes. Solubilization of the particulate membranes was achieved by the addition of 1% Triton X-100 and centrifugation at 200,000 × g for 120 min at room temperature. The supernatant was stored in aliquots at −70°C after removal of Triton X-100 by Bio-Beads SM-2 by the method of Holloway (11). The protein content was determined by the Lowry micromethod (16).

Iodination of Tyr-somatostatin. Both Tyr^1- and Tyr^{11}-somatostatin were iodinated using a modified chloramine-T method (7). Anion-exchange column chromatography using SM52 CMC (8 × 1 cm) was used for separation of the iodinated hormone from free iodine. Specific activity of about 350 mCi/mg or 700 Ci/mmol was achieved. The iodinated hormone was stored at −70°C and thawed only before use. All assays were performed within 10 days of iodination.

Binding Assay. Incubation was carried out in 0.5 ml 0.05 M Tris buffer with 0.025 M EDTA and 0.5% bovine serum albumin. The conditions of incubation, *i.e.* the pH of the buffer, time, temperature, and membrane concentration, were varied to

determine an optimal combination. Non-specific binding was determined as the binding observed in the presence of excess (10 μg/tube) unlabeled SRIF in the incubation media. Specific binding was calculated as the difference between nonspecific and total binding.

Concentrations of unlabeled SRIF from 0–5 μg/tube were incubated at pH 8 with 25 μg protein for 18 h in triplicate in order to determine the displacement of label by increasing concentrations of native hormone. After the incubation time was completed, 1 ml of chilled dextran-coated charcoal suspension (0.25% activated charcoal and 0.025% dextran T-70 in Tris/EDTA/albumin buffer) was added. Tubes were vortexed and left standing for 30 min at 4°C before centrifugation for 10 min at 2000 × g at 4°C. The supernatant was pipetted or poured off and counted. The pellet which represents the free 125I-Tyr-somatostatin was counted separately. The addition of toluene sulfonyl fluoride to the incubation medium slightly increased the stability of the preparation.

Assessment of degradation. Paper electrophoresis and column chromatography were used for assessment of label degradation after incubation with buffer or 25 μg membrane protein for 18 h at 4°C. Approximately 30 μl of solution containing labeled hormone was applied at the origin of paper strips and electrophoresed for 6–7 h at 275 watts on a Beckman paper electrophoresis cell. The paper strip was cut into 1-cm wide perpendicular pieces and each counted separately. Chromatography was performed using a 1 × 25 cm Bio-Gel P-30 column and the labeled hormone was eluted with 3 M acetate buffer at 4°C.

RESULTS

Specific binding of somatostatin to solubilized term placenta cell membranes was observed. This binding ranged from 5–21% within and between placentas with a mean of 10 ± 4% (SD), and was similar in all five placentas. Specific binding to particulate placenta cell membranes was not observed. Non specific binding with solubilized placenta cell membranes ranged from 6–21% the total counts with a mean of 13.5 ± 5.2% depending mostly on the integrity of the iodination product. Optimal specific binding was achieved at pH 8–9 and at an incubation temperature of 4°C for 18 h using 25 μg membrane protein per tube, as will be described below. These conditions were used for all of the assays except if otherwise indicated. Specific binding was similar in the premature and diabetic placentas.

Effect of membrane protein concentration on specific binding. The effect of membrane protein concentration per 0.5 ml of incubation volume on the specific binding of 125I-Tyr1-somatostatin to solubilized placenta cell membranes was evaluated. The amount of membrane protein varied from 2.5 to 200 μg/tube. Specific binding was maximal with 10–25 μg membrane protein/tube and decreased to nonexistent with very low (2.5 μg) or very high (200 μg) protein concentration per tube. Binding of the labeled hormone was tested over a range of 6,000–100,000 cpm, with optimal binding observed using approximately 25,000 cpm. Subsequent studies were performed using 25 μg membrane protein and 32.5 pg of labeled hormone per tube.

Effect of incubation time and temperature on specific binding. Specific binding was assessed at three different temperatures for the indicated time intervals. At 23°C, specific binding was minimal for the whole length of the incubation time period of 18 h. At 37°C, specific binding was consistently observed by 20 min, reached equilibrium by 160 min, and was stable for 1 additional h. Specific binding of 125I-Tyr1-somatostatin to solubilized placenta cell membranes at 4°C reaches equilibrium at 8 h and remains stable for 18 h.

Effect of pH on specific binding. The effect of pH on specific binding was assessed by varying the pH of the incubation media from 4 to 9 at 4°C for 18 h. In three separate experiments, specific binding was consistently lowest at pH 6 with optimum binding at pH 8 and 9.

Assessment of degradation of 125-I-Tyr-somatostatin. Degradation of the labeled hormone in the assay system assessed by paper electrophoresis and column chromatography. The results of the electrophoretic mobility of tracer, 125I-Tyr1-somatostatin incubated with increasing concentrations of membrane protein in the medium are depicted in Figure 1. Increased degradation of the labeled hormone, defined by the distance from the origin where the sample was applied, compared to control, was observed with increasing concentrations of solubilized membranes. Incubation with 25 μg of membrane protein for 18 h did not affect the migration pattern of 125I-Tyr1-somatostatin when compared to the pattern of labeled somatostatin incubated for the same time period without membrane protein. Similar results were obtained with 125I-Tyr11-somatostatin.

Following incubation of labeled Tyr1-somatostatin for 18 h with 25 μg membrane, most of the radioactivity eluted on column chromatography was in a large peak (*upper panel*, Fig. 2) comparable to the peak of radioactivity eluted on the same
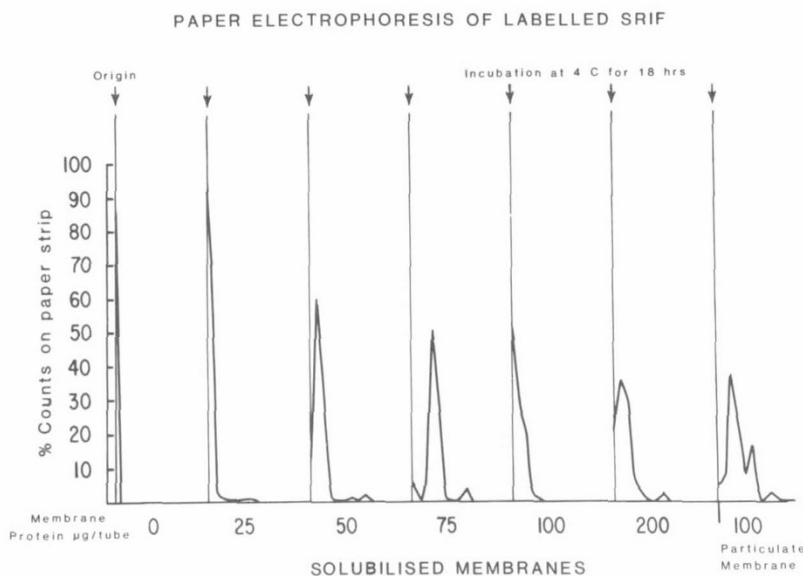

Fig. 1. Paper electrophoretic mobility patterns of 125I-Tyr1-somatostatin incubated with seven different membrane protein concentrations for 18 h at 4°C.

column from the labeled somatostatin incubated without membrane protein (*lower panel*, Fig. 2). The smaller peaks in the *upper panel* most probably represent membrane-bound labeled somatostatin and a small amount of degradated tracer bound to protein or protein aggregates. Note that the proportion of radioactivity eluted in this area coincides with the approximately 20% specific binding found using charcoal separation.

Competitive binding. Increasing concentrations of cyclic and linear native somatostatin displaced ^{125}I-Tyr^1-somatostatin. The three analogs Ala^5-somatostatin, Ala^8-somatostatin, and Ala^{11}-somatostatin also displaced ^{125}I-Tyr^1-somatostatin (Fig. 3). Similar displacement was shown with somatostatin 28. Scatchard plots of cyclic and linear somatostatin displacement of ^{125}I-Tyr^1-somatostatin are depicted in Figure 4. Similarly, cyclic somatostatin displaced ^{125}I-Tyr^{11}-somatostatin. The binding curve and

Scatchard plot are depicted in Figure 5. There was no displacement of ^{125}I-Tyr^{11}-somatostatin by other polypeptides such as insulin, growth hormone, and gonadotropin-releasing hormone, although cholecystokinin 10–20 displaced the label in parallel at a 50 times higher concentration while gastric inhibitory polypeptide and cholecystokinin 33 slightly displaced the binding (70%) at 500 times the concentration.

The capacity R_o and affinity and dissociation constants K_a and K_d of the competitive binding of cyclic and linear somatostatin and somatostatin analogs with the placental membranes displacing both Tyr^1- and Tyr^{11}-somatostatin were calculated. Higher affinity and dissociation constants were noticed with displacement of ^{125}I-Tyr^{11}-somatostatin than those of ^{125}I-Tyr^1-somatostatin. Somatostatin analog Ala^8-somatostatin had lower K_a and K_d in displacing either label in comparison with the other compounds displacing the same label.

Dissociation of ^{125}I-Tyr-somatostatin binding. Solubilized placental membranes, 25 μg protein/tube, were incubated with ^{125}I-Tyr^{11}-SRIF for 6 h at which time excess unlabeled SRIF was added. At the end of total 18 h of incubation, total binding was only 3% lower than that observed at 18 h when no cold hormone was added (Fig. 6), indicating either slow dissociation or irreversibility of binding. When cold hormone was added after 18-h incubation, no decrease in binding appeared after an additional 6 h of incubation.
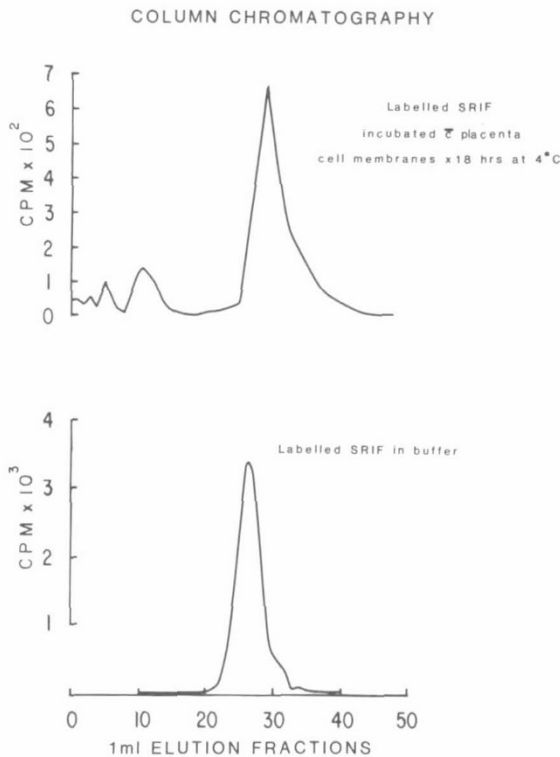
COLUMN CHROMATOGRAPHY

Fig. 2. Bio-Gel P-30 1 × 25 cm column chromatography of ^{125}I-Tyr^1-somatostatin buffer (*lower panel*) and after incubtion with 25 μg membrane protein for 18 h at 4°C (*upper panel*). Elution buffer was 3 M acetate at 4°C.
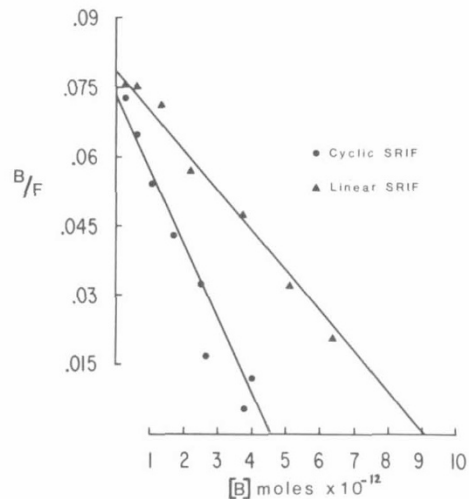

Fig. 4. Scatchard plots of displacement of ^{125}I-Tyr^1-somatostatin by cyclic and linear somatostatin.
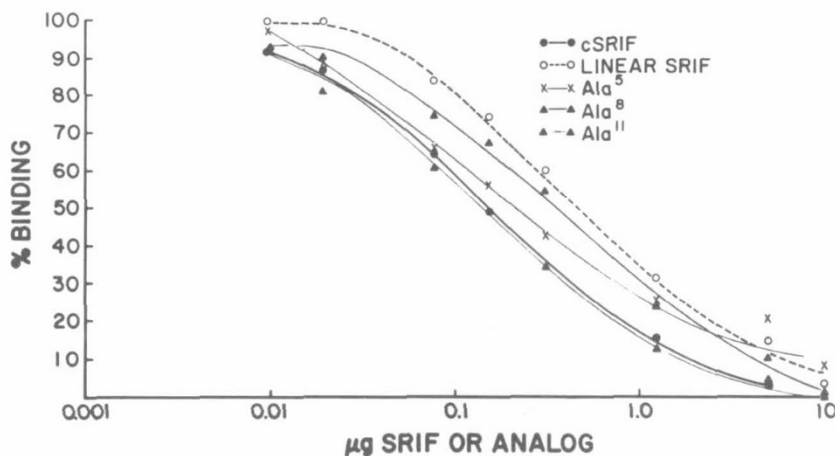

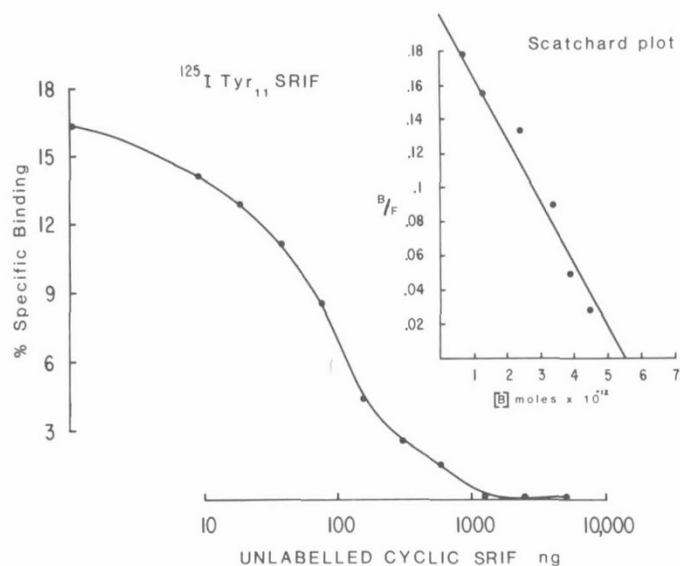Fig. 3. Displacement curves of ^{125}I-Tyr^1-somatostatin by cyclic and linear somatostatin.

Fig. 5. Displacement curve of ^{125}I-Tyr^{11}-somatostatin by cyclic somatostatin. The *abscissa* units are ng/tube. *Inset*, Scatchard plot of displacement data.
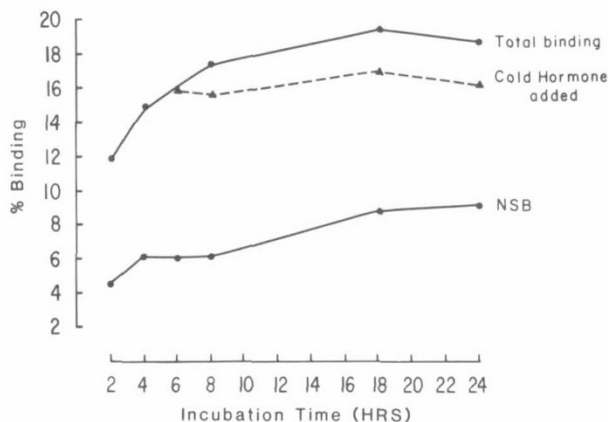

Fig. 6. Total and nonspecific binding and binding after cold hormone was added at 6-h incubation of ^{125}I-Tyr^{11}-somatostatin to 25 μg of placenta membrane protein incubated at 4°C for up to 24 h.

DISCUSSION

Binding sites for somatostatin have been identified in a number of tissues by demonstrating specific binding of labeled somatostatin to either intact cells or cell membrane preparations. It has been postulated that specific receptor sites exist only on those cells in which somatostatin has been shown to have a biological action (21). However, as is the case with insulin, there is a report of binding to mononuclear leukocytes (3), where there is no known biological action. In the present study, we described specific irreversible binding of somatostatin to placenta, a tissue in which somatostatin may exert a biological response.

Specific binding of somatostatin to the placental membranes was only identified after solubilization of the membranes with Triton X-100. Solubilization of the receptor eliminates proteolytic enzymes from the preparation, which are likely to degrade both the labeled and unlabeled hormone. As was demonstrated for insulin receptors on placenta cell membranes, this procedure does not change the binding characteristics of the receptors (9).

Little or no degradation was observed after incubation of labeled somatostatin with 25 μg of membrane protein using paper electrophoresis or column chromatography. As demonstrated by the faster moving label on paper electrophoresis,

increased degradation of somatostatin was observed with higher concentrations of membrane protein in the incubation media. This finding suggests that increasing concentrations of membrane protein degraded the iodinated polypeptide. Furthermore, the specific binding observed at the end of 18 h in the presence of 25 μg membrane protein in these studies represents that of the intact hormone and not a degradation product.

The optimal pH of this assay was found to be between 8 and 9, similar to that observed in somatostatin-binding assays using other tissues (14). Binding was shown at both 4 and 37°C with similar binding levels at equilibrium. Optimal time, temperature, and pH characteristics of this assay are similar to those demonstrated in other reports of somatostatin binding to membranes of other tissues.

We found similar displacement of both ^{125}I-Tyr^{1}- and Tyr^{11}-somatostatin binding by unlabeled cyclic somatostatin. Although cyclic somatostatin is the physiologic circulating form, similar displacement was shown using linear somatostatin. ^{125}I-Tyr^{11}-somatostatin had higher binding affinity than Tyr^{1}-somatostatin as has been observed in studies with other tissues (24).

Displacement of both ^{125}I-Tyr^{1}-somatostatin and ^{125}I-Tyr^{11}-somatostatin binding to the placenta cell membranes was also observed with three somatostatin analogs. The affinity constants of Ala^{5}-somatostatin and Ala^{11}-somatostatin were very similar to those for cyclic somatostatin, but lower affinity was observed for Ala^{8}-somatostatin. This finding coincides with the observation of Schonbrunn *et al.* (22) that substitution of residues 6–10 of somatostatin results in decreased affinity.

The affinity constant of somatostatin binding to placenta cell membranes is similar to that observed in membranes from the pituitary cells and in circulating blood cells (13, 21). Higher affinity was observed with binding in the GH$_4$C$_1$ cell line, brain synaptosomal membranes, adrenal capsular, and anterior pituitary membrane-rich particulate fractions (12, 21, 24). The two latter studies were performed with an iodinated analog which has been more active and more resistant to degradation than the native hormone and, therefore, may have different binding features.

Scatchard plots for somatostatin binding to placental membrane preparation were linear, indicating that these binding sites have the same affinity. Since linear and curvilinear Scatchard plots have been obtained in somatostatin binding assays, target tissues may vary in the number and type of receptors.

The lack of reversibility of somatostatin binding to placenta membranes is disturbing. Reversibility of binding has only occasionally been addressed in other somatostatin-binding studies in the literature. A high percentage of specific binding of the iodinated hormone was irreversible after incubation at either 37 or 4°C. It is possible that the experimental conditions we followed prevented the phenomenon. Degradation of the labeled peptide could be a reason for the irreversible binding observed, but displacement of the labeled hormone by analogs introduced prior to incubation was in accordance with their previously reported biological activity (4), and this would not occur if the tracer hormone were degraded. Therefore, we do not have a good explanation for these findings.

The significance of the irreversible specific binding of somatostatin to a solubilized placenta cell membrane preparation observed is unclear at the present time. A role for low affinity somatostatin-binding sites in human placenta remains hypothetical; an action of somatostatin on either the placental nutrient transport mechanism or on placental hormonal secretion has not yet been defined. It is possible that the placenta may be an easily obtainable source of tissue for the development of a radioreceptor assay for somatostatin which would, however, only be useful in studying the physiology of this hormone where it exists in high concentrations.

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Determination and Characterization of Immunoreactive Trypsin in Amniotic Fluid from Normal and Cystic Fibrosis Fetuses

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Summary

High concentrations of immunoreactive trypsin (IRT) in the blood, and low concentrations of trypsin activity in fecal specimens have been found in newborn infants with cystic fibrosis (CF). The amniotic fluid concentrations of IRT and of IRT in complex with α_1-antitrypsin (α_1AT) were studied in 39 samples taken in about the 17th gestational week, and in 7 samples taken because the mothers had previously given birth to children with CF. The midtrimester samples contained trypsin in complex with α_1AT in a concentration of 30–200 μg/liter, and small amounts of trypsinogen, 0–50 μg/liter. Three of four amniotic fluid samples from CF fetuses had very low concentrations of trypsin in complex with α_1AT (<10 μg/liter), and only small amounts of trypsinogen (<10 μg/liter). Further prospective studies are needed to ascertain whether the determination of IRT in amniotic fluid may be of use in prenatal diagnosis of CF.

Abbreviations

α_1AT, α_1-antitrypsin
CF, cystic fibrosis
g.w., gestational week
IRT, immunoreactive trypsin
RIA, radioimmunoassay