Megalin (gp330) Is an Endocytic Receptor for Thyroglobulin on Cultured Fisher Rat Thyroid Cells*

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We recently reported that megalin (gp330), an endocytic receptor found on the apical surface of thyroid cells, binds thyroglobulin (Tg) with high affinity in solid phase assays. Megalin-bound Tg was releasable by heparin. Here we show that Fisher rat thyroid (FRTL-5) cells, a differentiated rat thyroid cell line, can bind and endocytose Tg via megalin. We first demonstrated that FRTL-5 cells express megalin in a thyroid-stimulating hormone-dependent manner. Evidence of Tg binding to megalin on FRTL-5 cells and on an immortalized rat renal proximal tubule cell line (IRPT cells), was obtained by incubating the cells with 125I-Tg, followed by chemical cross-linking and immunoprecipitation of 125I-Tg with antibodies against megalin. To investigate cell binding further, we developed an assay in which cells were incubated with unlabeled Tg at 4 °C, followed by incubation with heparin, which released almost all of the cell-bound Tg into the medium. In solid phase experiments designed to illuminate the mechanism of heparin release, we demonstrated that Tg is a heparin-binding protein, as are several megalin ligands. The amount of Tg released by heparin from FRTL-5 and IRPT cells, measured by enzyme-linked immunosorbent assay (ELISA), was markedly reduced by two megalin competitors, receptor-associated protein (RAP) and 1H2 (monoclonal antibody against megalin), indicating that much of the Tg released by heparin had been bound to megalin (∼60–80%). The amount inhibited by RAP was considered to represent specific binding to megalin, which was saturable and of high affinity (Kd = 11.2 nM). Tg endocytosis by FRTL-5 and IRPT cells was demonstrated in experiments in which cells were incubated with unlabeled Tg at 37 °C, followed by heparin to remove cell-bound Tg. The amount of Tg internalized (measured by ELISA in the cell lysates) was reduced by RAP and 1H2, indicating that Tg endocytosis is partially mediated by megalin.

Thyroglobulin (Tg)1 is synthesized in thyocytes and released into the follicle lumen, where it is stored as the major component of the colloid (1, 2). Post-transitional modifications of Tg that occur mainly at the cell-colloid interface lead to forms that are iodine-rich and that contain the thyroid hormones T4 and T3 (mature Tg). Hormone secretion requires uptake of Tg by thyocytes, with transport to lysosomes, where proteolytic cleavage leads to release of the hormones from mature Tg molecules (1). Internalization of Tg may result from pseudopod ingestion under certain circumstances, such as intense, acute stimulation by the thyrotropic hormone (TSH), but micropinocytosis (vesicular internalization) is thought to be the usual route (1–3). There is evidence that micropinocytosis of Tg can take place both by nonselective fluid phase uptake and receptor-mediated endocytosis, but the relative importance of these two mechanisms is uncertain (1). Although evidence has been obtained of low affinity receptors for Tg on thyroid cells, a receptor capable of mediating Tg endocytosis has not been fully characterized (1–12).

We have previously obtained evidence suggesting that megalin (gp330) may function as a receptor for Tg (13). Megalin (gp330) is a member of the LDL receptor family (14, 15) and has been shown to bind multiple, unrelated ligands and to mediate endocytosis of ligands via coated pits, leading to delivery of ligands to lysosomes, where degradation occurs (16–18). In immunohistochemical studies, megalin has been found principally on the apical surface of a restricted group of absorptive epithelial cells, including renal proximal tubule cells, epididymal cells, type II pneumocytes, and thyroid epithelial cells (19, 20). Based on the assumption that physiological ligands of megalin may be identified by consideration of the composition of fluids to which it is exposed in various organs (21), we postulated that megalin on thyocytes serves as a receptor for Tg. In support of this possibility we demonstrated in solid phase assays that purified rat megalin binds to rat Tg with high affinity (13). Binding was inhibited by several known competitors of megalin, including the receptor-associated protein (RAP), antibodies to megalin, and heparin, which in addition dissociated Tg bound to megalin (13). In the present study we have investigated FRTL-5 cells to determine whether they express megalin and if they are capable of binding and internalizing Tg via megalin. FRTL-5 cells, an established rat thyroid cell line, exhibit a number of thyroid-specific functions in a TSH-dependent manner (22, 23). Here we show that FRTL-5 cells do express megalin and that they can bind and endocytose Tg via megalin.

EXPERIMENTAL PROCEDURES

Materials—Tg was purified from rat thyroids by ammonium sulfate precipitation and column fractionation, as described previously (24).

lipoprotein receptor-related protein; ELISA, enzyme-linked immunosorbent assay; ALP, alkaline phosphatase; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; TBS, Tris-buffered saline.

1 The abbreviation used are: Tg, thyroglobulin; LDL, low density lipoprotein; TSH, thyrotropic-stimulating hormone; RAP, receptor-associated protein; GST, glutathione S-transferase; FRTL-5 cells, Fisher rat thyroid cells; CHO cells, Chinese hamster ovary cells; IRPT cells, immortalized rat proximal tubule cells; OVA, ovalbumin; LR, low density

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The Tg preparations were analyzed by Western blotting, using a rabbit anti-human Tg antibody cross-reactive with Tg from other species (Axel-Westbury, NY). Two bands were seen at about 330 and 660 kDa, corresponding to the monomeric and the dimeric forms of Tg. The RAP was used in the form of a glutathione S-transferase (GST) fusion protein. HDL (human high density lipoprotein) was used to precipitate Tg from Becton Dickinson (Mountain View, CA). The Tg preparations were analyzed by Western blotting, using a rabbit anti-human Tg antibody (1:500), or goat antihuman Tg antibody (1:500 mg/ml). After 4 h of incubation at 4 °C, the cells were washed twice with ice-cold PBS to remove nonspecifically bound proteins and then incubated for 1 h at 4 °C with ice-cold heparin (10–100 units/ml in PBS), to release receptor-bound proteins from the cell surface. In certain experiments, to measure total cell-bound Tg, cell lysates were prepared by treating the cells with ice-cold H2O on ice, immediately after incubation with Tg at 4 °C. The amounts of Tg, RAP-GST or GST in the heparin wash or in the cell lysates were measured by ELISA. For this purpose, 96-well microtiter plates were coated overnight at 4 °C with the heparin wash or with the cell lysates, blocked with bovine serum albumin (Sigma) and incubated with rabbit anti-human Tg (1:500), anti-RAP (20 μg/ml), or goat anti-GST (1:1000) antibodies, followed by ALP-conjugated goat anti-rabbit IgG (200 μg/ml) and 1% OVA. Absorbance was determined at 405 nm. For inhibition experiments, biotin-labeled hepargin-albumin–albumin complex (Bio-Rad) was added to the plates together with one of the following: RAP-GST (200 μg/ml), 1H2 (200 μg/ml) or 1H2 (200 μg/ml) and mouse anti-GST (1:500). After 6 h of incubation the cells were washed twice with PBS and incubated with ice-cold heparin, as described above, to remove cell surface bound proteins, which were measured by ELISA. The cells were then lysed to measure internalized Tg, RAP-GST, or GST, using deionized H2O on ice. The amount of cell protein was measured in an aliquot of the cell lysate. Internalized Tg, RAP-GST or GST were measured in the cell lysates by ELISA, as described above.

**RESULTS**

**Expression of Megalin by Cultured Cells—** As shown in Fig. 1A, FRFTL-5 cells were found to express megalin by FACS analysis, using either the mouse monoclonal (1H2) or the rabbit monoclonal (A55) anti-megalin antibodies. In addition, immunoperoxidase staining with 1H2 demonstrated surface megalin (Diaminobenzidin (Sigma) was used for the color reaction.

**Cross-linking Experiments—** Cells were incubated with serum-free Dulbecco’s modified Eagle’s medium for 1 h at 37 °C and then blocked in 0.5% ovalbumin (OVA, Sigma), 25 mM Trit, 150 mM NaCl, 5 mM CaCl2, 0.5 mM MgCl2, pH 8.0, for 1 h at 4 °C. Cells were then incubated with 10 μg/ml of Tg (1:500 mg/ml) for 3 h at 4 °C, by incubating with the homobifunctional cross-linker 3’3’-dithiobis(sulfosuccinimidyl) propionate (Pierce) (0.5 mg/ml in PBS) for 30 min at 4 °C. After washing with TBS, cells were lysed with 1% Triton X-100, 1% deoxycholate and briefly sonicated. Cell lysates (500 μg) were immunoprecipitated with the rabbit anti-megalin antibody (A55 anti-serum, 1:200) or with the rabbit anti-human Tg antibody (1:500) and analyzed by Western blotting, using protein A beads (Pierce). The precipitates were separated by 4–16% SDS-polyacrylamide gel electrophoresis under nonreducing conditions and analyzed by autoradiography. Radioactivity in the cell lysates and in the precipitates was counted with a γ counter.

**Release of Cell Bound Exogenous Tg by Heparin—** Cells were cultured in 96-well tissue culture plates until 80–100% confluence was reached. Cells were incubated with unlabeled Tg or, as controls, with RAP-GST or GST in Coon’s F12 medium containing 5 mM CaCl2, 0.5 mM MgCl2, and 0.5% OVA. For inhibition experiments, FRFTL-5 and IRPT cells were incubated with Tg alone, or in the presence of megalin inhibitors, namely RAP-GST (200 μg/ml) or 1H2 (200 μg/ml) or, as controls, with GST (200 μg/ml) or normal mouse IgG (200 μg/ml). After 4 h of incubation at 4 °C, the cells were washed twice with ice-cold PBS to remove nonspecifically bound proteins and then incubated for 1 h at 4 °C with ice-cold heparin (10–100 units/ml in PBS), to release receptor-bound proteins from the cell surface. In certain experiments, to measure total cell-bound Tg, cell lysates were prepared by treating the cells with ice-cold H2O on ice, immediately after incubation with Tg at 4 °C. The amounts of Tg, RAP-GST or GST in the heparin wash or in the cell lysates were measured by ELISA. For this purpose, 96-well microtiter plates were coated overnight at 4 °C with the heparin wash or with the cell lysates, blocked with bovine serum albumin (Sigma) and incubated with rabbit anti-human Tg (1:500), rabbit anti-RAP (20 μg/ml), or goat anti-GST (1:1000) antibodies, followed by ALP-conjugated goat anti-rabbit IgG (200 μg/ml) and 1% OVA. Absorbance was determined at 405 nm. For inhibition experiments, biotin-labeled hepargin-albumin–albumin complex (Bio-Rad) was added to the plates together with one of the following: RAP-GST (200 μg/ml), 1H2 (200 μg/ml) or, as controls, GST (200 μg/ml) or normal mouse IgG (200 μg/ml). After 6 h of incubation the cells were washed twice with PBS and incubated with ice-cold heparin, as described above, to remove cell surface bound proteins, which were measured by ELISA. The cells were then lysed to measure internalized Tg, RAP-GST, or GST, using deionized H2O on ice. The amount of cell protein was measured in an aliquot of the cell lysate. Internalized Tg, RAP-GST or GST were measured in the cell lysates by ELISA, as described above.
on FRTL-5 cells (Fig. 1B). Megalin was also detected in FRTL-5 cell extracts by Western blotting (Fig. 1C). In agreement with previous reports (30, 31), IRPT cells were found to express megalin by FACS analysis, whereas megalin was not detected on CHO cells (not shown). Because LRPs bind many of the same ligands as megalin, we studied its expression on FRTL-5 cells. Both A55 and anti-Tg antibodies precipitated a very high molecular mass material. The figure is representative of one of three experiments performed.

Expression of Megalin by FRTL-5 Cells and TSH Dependence—To investigate whether megalin expression by FRTL-5 cells is regulated by TSH, cells were cultured for 24 h in medium containing TSH (10 milliunits/ml), followed by 48 h in fresh medium with TSH. The staining for megalin ranged from IRPT cells was 11.39%. The results are consistent with the presence of Tg receptors on thyroid cells in addition to megalin, as suggested by previous studies (1–12). The proportion of 125I-Tg precipitated by the anti-megalin antibody was 8.48% from FRTL-5 cells and 11.81% from IRPT cells.

Cross-linking and Immunoprecipitation of 125I-Tg with Megalin—In experiments designed to obtain evidence of Tg binding to cell surface megalin, FRTL-5 and IRPT cells were incubated with 125I-Tg at 4 °C, followed by cross-linking and immunoprecipitation with anti-Tg or anti-megalin antibodies. As shown in Fig. 3A, both anti-Tg and anti-megalin antibodies produced a high molecular mass band at the same size, indicating Tg bound to megalin on the cell surface. Another band at approximately 50 kDa was produced by the anti-megalin antibody and not by the anti-Tg antibody. The identity of this product is unknown.

As shown in Fig. 3B, the proportion of 125I-Tg precipitated by the rabbit anti-Tg antibody from FRTL-5 cells was 19.9% of the total amount of 125I-Tg in the cell lysates, whereas that obtained from IRPT cells was 11.39%. The results are consistent with the presence of Tg receptors on thyroid cells in addition to megalin, as suggested by previous studies (1–12). The proportion of 125I-Tg precipitated by the anti-megalin antibody was 8.48% from FRTL-5 cells and 11.81% from IRPT cells.

Binding of Tg to Megalin on Cultured Cells, Assessed by Heparin Release—We previously showed that heparin dissociates Tg from megalin in solid phase assays (13). Here we studied the ability of heparin to dissociate Tg bound to megalin on cells, to be used as a measure of cell bound Tg. Cells were incubated with unlabeled Tg at 4 °C, followed by incubation with heparin. In addition, some cells were incubated with RAP (used as a RAP-GST fusion protein), as a positive megalin binding control, or with GST, as a negative control, before heparin treatment. The amounts of Tg, RAP-GST, and GST in the heparin wash were measured by ELISA.

As shown in Fig. 4A, heparin was found to release Tg and...
RAP-GST from FRTL-5 and IRPT cells. Although FRTL-5 cells are capable of synthesizing Tg (22, 23), the amount of Tg released by heparin from FRTL-5 cells incubated in medium lacking Tg was negligible. RAP-GST was also released from CHO cells, which express LRP (32) but not megalin. The amount of Tg released by heparin from CHO cells was negligible as compared with FRTL-5 and IRPT cells (Fig. 4A). No release of GST was observed from FRTL-5, IRPT, or CHO cells. The amount of Tg released from FRTL-5 cells by heparin was dependent on its concentration, with linear increase up to 100 units/ml, the highest concentration used (not shown).

Inhibition experiments showed that much of the exogenous Tg released by heparin from FRTL-5 and IRPT cells was bound to megalin on the cell surface. Thus, when the cells were incubated with Tg plus either of two megalin competitors, namely RAP-GST or 1H2 (monoclonal antibody against megalin), the amount of Tg released by heparin was markedly reduced (Fig. 4B). The mean inhibition produced by RAP-GST was 84% in FRTL-5 cells and 78% in IRPT cells. The mean inhibition produced by 1H2 was 62% in FRTL-5 cells and 73% in IRPT cells. No reduction of heparin-releasable Tg was produced by co-incubation of the cells with Tg plus GST or mouse IgG, used as negative controls.

To measure the proportion of total cell surface-bound Tg that was released by heparin, experiments were performed in which the amount of Tg released by heparin was compared with the amount of Tg found in cell lysates from FRTL-5 or IRPT cells that were not subjected to heparin treatment but immediately lysed after 4 h of incubation with Tg at 4 °C. Because at this temperature no internalization occurs, the amount of Tg found in the cell lysates represents only exogenous Tg bound to the cell surface, minus a negligible amount (~1%) contributed by endogenous Tg, as found in FRTL-5 cells not incubated with exogenous Tg. This amount was similar to that found in the heparin wash of FRTL-5 cells incubated with medium lacking Tg.

As shown in Fig. 5A (left bars), almost all (~97%) of the total cell-bound Tg was released by heparin both from FRTL-5 and IRPT cells, indicating that heparin-releasable Tg can be considered a measure of total cell surface-bound Tg. Co-incubation of cells with Tg plus the megalin competitors RAP-GST or 1H2 resulted in reduced amounts of total-cell-bound Tg (Fig. 5A, right bars), showing that much of the Tg was bound to megalin. No reduction of total cell-bound Tg was produced by co-incubation of the cells with Tg plus GST or mouse IgG, used as negative controls. The percentage of inhibition of total cell-bound Tg obtained with RAP-GST or 1H2 was similar to the percentage of inhibition of heparin-releasable Tg produced by the same competitors (Fig. 4B). Therefore, inhibition of heparin-releasable Tg by megalin competitors can be considered as a measure of megalin-bound Tg, which ranged from ~60 to ~80%, depending on the cell type and on the competitor used.

As shown in Fig. 5B, when FRTL-5 cells were incubated with increasing concentrations of unlabeled Tg, the total amount of cell surface-bound Tg released by heparin was saturable, as is typical of receptor binding. When cells were incubated with increasing concentrations of unlabeled Tg plus a constant concentration of RAP-GST (200 µg/ml), inhibition of heparin-releasable Tg was obtained, ranging from ~80% at low Tg concentrations to ~60% at high Tg concentrations. The amount of Tg bound in the presence of RAP-GST was considered to be unrelated to megalin, and this showed some degree of saturation, suggesting the presence of Tg receptors, in addition to megalin, on FRTL-5 cells. The amount of Tg binding that was inhibited by RAP-GST (obtained by subtracting from the total amount of heparin releasable Tg the amount released in the presence of RAP-GST) was considered to be specific binding to megalin, and this was more highly saturable. The equilibrium dissociation constant ($K_d$) of Tg binding to FRTL-5 cells was calculated according to the method of Furchgott (33), which is based on comparison between the binding of an unlabeled protein to a receptor in the presence or in the absence of a known competitor of the receptor, in this case RAP-GST. The calculated $K_d$ values in the three experiments performed ranged from 7.8 to 14.3 nM (mean 11.2 ± 3.0 nM), indicating a high affinity interaction between megalin and Tg on FRTL-5 cells.

**Binding of Heparin to Tg**—To help understand the mechanism by which heparin competes with megalin for Tg binding, and in view of the knowledge that megalin does not bind to heparin (26, 34), solid phase binding assays were performed to determine whether Tg is a heparin-binding protein. Tg-coated wells were incubated with biotin-labeled heparin or, as a control, with biotin-labeled albumin. As shown in Fig. 6, biotin-labeled heparin bound to Tg-coated wells (Fig. 6, line 1) but not to ovalbumin-coated wells (Fig. 6, line 4). No binding of biotin-labeled albumin was observed (Fig. 6, line 3). Binding of biotin-labeled heparin-albumin to Tg was saturable and was almost completely inhibited by co-incubation with unlabeled heparin (Fig. 6, line 2).

**Uptake of Tg by Cultured Cells Is Partially Mediated by Megalin**—Experiments were performed by incubating cells at
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Fig. 5. A, comparison of total cell surface-bound Tg with heparin-releasable Tg from FRTL-5 and IRPT cells and proportion of megalin-bound Tg. Cells were incubated for 4 h at 4 °C, with unlabeled Tg (10 μg/ml), alone or in the presence of RAP-GST or mouse monoclonal anti-megalin antibody (1H2). GST and normal mouse IgG were used as controls. Total cell-bound Tg was measured by ELISA in cell lysates obtained by treatment with the cells with ice-cold H2O immediately after incubation with Tg at 4 °C. Tg released by heparin was measured in the heparin wash after treating the cells with heparin (100 units/ml). Values were normalized for the amount of protein in the cell lysate. Similar amounts of Tg were found in the cell lysate and in the heparin wash of FRTL-5 cells incubated with medium lacking Tg, which were negligible and were subtracted as background. Results are expressed as the means ± S.E. obtained in three experiments. B, saturation of Tg release by heparin from FRTL-5 cells. Cells were incubated with Tg at various concentrations, alone or in the presence of RAP-GST, followed by heparin (100 units/ml). The amount of Tg released by heparin that was inhibited by RAP-GST was considered as a measure of specific binding of Tg to megalin and was calculated by subtracting the amount released by heparin in the absence of RAP-GST from the amount released by heparin in the presence of RAP-GST. The amount of Tg in the heparin wash of FRTL-5 cells incubated in medium lacking Tg was subtracted, as background. The figure is representative of one of three experiments performed.

Fig. 6. Binding of heparin to Tg. 96-well microtiter plates were coated overnight at 4 °C with purified rat Tg or, as a control, with OVA and incubated with biotin-labeled heparin or, as a control, with biotin-labeled albumin, followed by ALP-conjugated streptavidin. Absorbance (OD) was determined at 405 nm. 1, binding of biotin-labeled heparin to Tg-coated wells. 2, binding of biotin-labeled heparin to Tg-coated wells in the presence of 500 units/ml of unlabeled heparin. 3, binding of biotin-labeled albumin to Tg-coated wells. 4, binding of biotin-labeled heparin to OVA-coated wells. The figure is representative of one of three experiments performed.

DISCUSSION

In the present study we show that megalin is a receptor for Tg endocytosis on cultured thyroid cells. We first demonstrated that FRTL-5 cells, a well studied established rat thyroid cell line (22, 23), express megalin when cultured in standard medium containing the thyrotropic hormone (TSH). As shown by FACS analysis and immunoperoxidase staining, megalin was present on the cell surface. FRTL-5 cells are known to maintain several specific thyroid functions, most of which are TSH-dependent (22, 23). Therefore, the demonstration that megalin expression by FRTL-5 cells is TSH-dependent indirectly supports a thyroid-related function of megalin. In particular, because TSH provides a signal for Tg internalization (1–3), the finding suggests a role of megalin as an endocytic receptor.

Direct evidence of binding of Tg to megalin on FRTL-5 cells as well as on IRPT cells, a rat renal proximal tubule cell line that expresses abundant megalin (30, 31), was provided by experiments in which cells were incubated with 125I-labeled Tg followed by cross-linking and incubation of the cell extracts with antibodies against megalin, which resulted in co-immunoprecipitation of 125I-Tg. In other experiments, binding of Tg to megalin on FRTL-5 and IRPT cells was demonstrated by incubation of cells with unlabeled Tg at 4 °C, followed by treatment with heparin, which released Tg into the medium, as detected by ELISA. Because almost all (~97%) of the total cell-bound Tg was released by heparin, the amount of heparin-releasable Tg can be considered as a measure of cell surface-
bound Tg.

We previously showed in solid phase experiments that heparin dissociates Tg from purified megalin (13). Goldstein and colleagues (27) had shown earlier that heparin releases the LDL from its receptor on cultured fibroblasts and used this finding to measure the amount of binding of LDL to the LDL receptor. However, it is known that heparin can release molecules not only from members of the LDL receptor family but also from heparan sulfate proteoglycans (35), which are expressed in many cell types, including FRTL-5 cells (36). Nevertheless, in our experiments with FRTL-5 cells we obtained compelling evidence that most of the Tg released by heparin had been bound to megalin. Thus, when cells were incubated with Tg plus the monoclonal anti-megalin antibody 1H2 or RAP, there was a 60–80% reduction in heparin releasable Tg as well as in total cell-bound Tg, which represents the proportion of megalin-bound Tg. 1H2 is entirely specific for Tg binding to megalin on FRTL-5 cells is saturable and of high affinity (Kd < 11.2 nM). However, the finding that binding not related to megalin (noninhibitable by RAP) also showed some degree of saturation supports the existence, in addition to megalin, of other Tg receptors on thyroid cells, as suggested in other studies (1–12). This possibility is also supported by the finding that in co-immunoprecipitation experiments a higher proportion of Tg was precipitated by the anti-Tg antibody from FRTL-5 cells than from IRPT cells.

Based on the finding that heparin dissociates Tg from megalin, we postulated that Tg is a heparin-binding protein, because megalin itself does not bind to heparin (26, 34) and because several megalin ligands are heparin-binding proteins (26). Indeed, this prediction was confirmed by solid phase assays, which showed specific binding of Tg to heparin. This observation suggests that regions rich in positively charged amino acid residues (arginine and lysine) in the Tg molecule may contribute to its binding to megalin, as has been demonstrated for binding of certain other megalin ligands, including aprotinin and polybasic drugs (43).

Experiments in which FRTL-5 and IRPT cells were incubated with unlabeled Tg at 37 °C, followed by heparin treatment to remove cell-bound Tg, showed that megalin can mediate Tg endocytosis. The detection of Tg in FRTL-5 cell lysates clearly showed that exogenous Tg had been internalized, because Tg of endogenous origin was considerably lower, as found in lysates from cells incubated in medium lacking Tg. Furthermore, the demonstration that almost all of the cell surface-bound Tg was released by heparin provides evidence that the amount of Tg found in the cell lysates represented only Tg that had been internalized. Moreover, because Tg was measured by ELISA, the amount of Tg internalized may have been underestimated, because Tg degradation during the course of the incubation should cause some loss of immunoreactivity.

Inhibition experiments provided evidence that a certain amount of Tg uptake is mediated by megalin. Thus, internalization of Tg by FRTL-5 and IRPT cells was appreciably reduced when cells were co-incubated with exogenous Tg plus RAP or 1H2. Furthermore, we obtained evidence against the possibility that reduction of Tg uptake in FRTL-5 and IRPT cells by megalin competitors resulted merely from lowering the amount of Tg bound to the cells. Thus, the ratio of internalized Tg to cell surface-bound Tg was reduced by RAP and 1H2.

The inhibition of Tg uptake produced by megalin competitors in FRTL-5 cells was not complete (~50%), suggesting that, in addition to megalin, other mechanisms are responsible for Tg endocytosis, as suggested by previous studies (1–12, 44). The finding that less inhibition of uptake was produced by megalin competitors in FRTL-5 cells than in IRPT cells (~70%) suggests that the contribution of megalin to Tg uptake is greater in IRPT cells. As noted earlier, there is evidence that micropinocytosis of Tg by thyrocytes occurs both through fluid phase uptake and receptor-mediated endocytosis (1–12). However, despite extensive investigations, a specific receptor shown to have a major role in Tg uptake by thyrocytes has not previously been characterized. Consiglio et al. (4, 5) showed the existence of a specific binding site for asialogalacto-Tg in thyroid membrane preparations as well as in cultured thyrocytes, and this was confirmed by others (6). The receptor was identified in porcine...
thyroid membranes as a 45-kDa protein, which was suggested to be involved mainly in Tg recycling (7). More recently, Lemansky and Herzog (11) performed a study using porcine thyroid follicles, designed to investigate the role of mannose-6-phosphate receptors in Tg endocytosis through interaction with mannose-6 recognition markers on Tg N-linked glycans. Although they failed to show that mannose-6-phosphate receptors are responsible for Tg endocytosis, they did obtain evidence of specific low affinity binding sites on the apical surface of thyrocytes involved in Tg endocytosis. However, the responsible receptor was not identified. In another study, Giraud et al. (10) obtained evidence of selective, moderately high affinity binding of Tg to thyrocytes involved in Tg endocytosis. However, the responders are responsible for Tg endocytosis, they did obtain evidence of specific low affinity binding sites on the apical surface of thyrocytes involved in Tg endocytosis. However, the responsible receptor was not identified.

In summary, evidence obtained in our previous (13) and present studies supports the conclusion that megalin can function as a receptor on thyrocytes capable of mediating binding and uptake of Tg. Receptors other than megalin probably contribute to this process and fluid phase pinocytosis may play a major role (1–12, 44). In any case, the ability of megalin to bind Tg with high affinity raises interesting questions about its function in vivo. High affinity receptors serve to mediate endocytosis of ligands that are generally present in low concentrations in extracellular fluids and thus serve to compete effectively with fluid phase uptake. However, Tg in the colloid is very highly concentrated (100–200 mg/ml), which is consistent with the notion that nonspecific fluid phase uptake is the major mechanism for Tg endocytosis and hormone release (1). The function of a high affinity receptor for Tg on thyroid cells may therefore be to regulate the extent of endocytosis only under special circumstances. High affinity receptor binding should lead to an increase of Tg endocytosis, which would be expected to result in delivery to lysosomes, with hormone release. However, it is also possible that the receptor could divert Tg from the usual endocytic pathway, as through recycling or transcytosis. Further studies are needed to define the role of megalin in thyroid hormone release.

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