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Na^+–I^- symport activity is present in membrane vesicles from thyrotropin-deprived non-I^--transporting cultured thyroid cells

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ABSTRACT **The active accumulation of I^- in the thyroid gland is mediated by the Na^+–I^- symporter and driven by the Na^+ gradient generated by the Na^+/K^+-ATPase. Thyrotropin (TSH) stimulates thyroidal I^- accumulation. Rat thyroid-derived FRTL-5 cells require TSH to accumulate I^-. TSH withdrawal for over 7 days results in complete loss of Na^+–I^- symport activity in these cells [Weiss, S. J., Philp, N. J. and Grollman, E. F. (1984) *Endocrinology* 114, 1090–1098]. Surprisingly, membrane vesicles prepared from FRTL-5 cells maintained in TSH-free medium [TSH(−) cells] accumulate I^-, suggesting that the absence of Na^+–I^- symport activity in TSH(−) cells cannot be due solely to a decrease in the biosynthesis of either the symporter or a putative activating factor. This finding indicates that the Na^+–I^- symporter is present, probably in an inactive state, in TSH(−) cells despite their lack of Na^+–I^- symport activity. Na^+–I^- symport activity in thyroid membrane vesicles is enhanced when conditions for vesicle preparation favor proteolysis. Subcellular fractionation studies in both TSH(+) and TSH(−) cells show that Na^+–I^- symport activity is mostly associated with fractions enriched in plasma membrane rather than in intracellular membranes, suggesting that the Na^+–I^- symporter may constitutively reside in the plasma membrane and may be activated by TSH.**

I^- accumulation in the thyroid gland is the first step in the biosynthesis of triiodothyronine (T_3) and thyroxine (T_4) (1–3). The Na^+–I^- symporter mediates I^- transport across the basolateral membrane of thyroid follicular cells (4). Studies in thyroid slices (5), tissue culture cells (6–8), and membrane vesicles (MV) (9–11) and on the Na^+–I^- symporter expressed in *Xenopus laevis* oocytes (12) indicate that this protein couples the inward translocation of Na^+ down its electrochemical gradient to the simultaneous inward "uphill" translocation of I^- against its electrochemical gradient. The Na^+ gradient acting as the driving force for I^- uptake is generated by the Na^+/K^+-ATPase.

Thyrotropin (thyroid-stimulating hormone, TSH) released from the pituitary regulates many thyroid functions, including the stimulation of Na^+–I^- symport. The rat thyroid-derived cell line FRTL-5 (13) requires TSH for growth. However, these cells can be maintained in TSH-free medium [TSH(−) cells] for as long as 14 days without loss of viability (7). The transfer of FRTL-5 cells to TSH-free medium leads first to a rapid decline of intracellular cAMP (within 3 hr) and then to a total loss of Na^+–I^- symport activity after 7 days (7, 8). Readdition of TSH to the medium restores Na^+–I^- symport activity after a latency of 12–24 hr. Stimulation of Na^+–I^- symport by TSH is blocked by actinomycin D and cycloheximide (8). However, actinomycin D paradoxically increases TSH-stimulated I^- accumulation when added during the late phase of protein synthesis (14).

It has generally been accepted that TSH stimulates Na^+–I^- symport activity solely by promoting the biosynthesis of either the Na^+–I^- symporter or an activating factor (8, 15). Conversely, the absence of Na^+–I^- symport activity in TSH(−) FRTL-5 cells has been attributed to a decrease in the biosynthesis of the Na^+–I^- symporter or an activating factor. However, we report that MV prepared from TSH(−) cells surprisingly exhibit I^- accumulation, indicating that the Na^+–I^- symporter is present in these cells despite their lack of I^- transport activity. Preparation of MV from both TSH(+) and TSH(−) cells under conditions that promote proteolysis resulted in enhanced Na^+–I^- symport. The results are consistent with a model in which the Na^+–I^- symporter constitutively resides in the plasma membrane (PM) and is activated by TSH.

EXPERIMENTAL PROCEDURES

Preparation of MV. FRTL-5 cells were grown as described (7, 10). MV were prepared by a modification of a published procedure (16). In brief, TSH(+) or TSH(−) FRTL-5 cells were washed, harvested, and resuspended in ice-cold 250 mM sucrose/1 mM EGTA/10 mM Hepes·KOH, pH 7.5, containing aprotinin (90 μg/ml), benzamidine (1 mM), leupeptin (4 μg/ml), phenylmethanesulfonyl fluoride (0.8 mM), pepstatin (4 μg/ml), and E-64 (10 μg/ml) and were disrupted with a motor-driven Teflon-pestle homogenizer (25 strokes). The homogenate was centrifuged twice at 500 × *g* for 15 min at 4°C. The supernatant was centrifuged at 100,000 × *g* for 1 hr at 4°C. The pellet was resuspended in ice-cold 250 mM sucrose/1 mM $MgCl_2$/10 mM Hepes·KOH, pH 7.5, aliquoted, and stored in liquid nitrogen. MV from Sprague–Dawley rat thyroids were prepared as described above, except that a glass homogenizer was used.

Transport Assays. I^- transport assays in FRTL-5 cells and in MV were performed as reported (10, 14). The Na^+/K^+-ATPase was assayed by $^{86}Rb^+$ uptake (17).

Determination of MV Internal Volume. Intravesicular volume was determined by methyl α-D-[U-^{14}C]glucopyranoside equilibration (18). MV (50 μg of protein) were incubated at 25°C with 1 and 10 mM methyl α-D-[U-^{14}C]glucopyranoside (7 and 46 mCi/mmol, respectively; 1 mCi = 37 MBq) to reach saturation. Reactions were terminated with 4 ml of ice-cold quenching solution (250 mM KCl/1 mM Tris·HCl, pH 7.5), followed by rapid filtration through nitrocellulose filters (0.45-μm pores). Filters were washed twice with an additional 4 ml of quenching solution. Radioactivity retained by MV was determined by liquid scintillation spectrometry.

Immunoblot Analyses. FRTL-5 MV (20–40 μg of protein) were subjected to SDS/9% PAGE (19). Polypeptides were electrotransferred to nitrocellulose overnight at 60 mA in the original buffer (20) with the addition of 0.05% lithium dodecyl sulfate. Nitrocellulose was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS: 137 mM NaCl/2.4 mM KCl/25

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Abbreviations: ER, endoplasmic reticulum; MV, membrane vesicle(s); PM, plasma membrane; PNS, postnuclear supernatant; TSH, thyroid-stimulating hormone (thyrotropin).

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mM Tris, pH 8.0) for 1 hr, washed with TBS/1% Tween 20 once for 15 min and three times for 5 min, and incubated with the corresponding antibody for the indicated times. Nitrocellulose was washed again as indicated above and incubated with a 1:1500 dilution of a horseradish peroxidase-linked donkey anti-rabbit IgG (Amersham) for 1 hr. Polypeptides were visualized by the enhanced chemiluminescence (ECL) Western blot detection system (Amersham).

Subcellular Fractionation. TSH(+) and TSH(−) FRTL-5 cells were fractionated by a modification of two protocols (21, 22). FRTL-5 cells from at least 15 dishes (150-mm diameter) were harvested and homogenized in buffer A (250 mM sucrose/1 mM EGTA/20 mM Hepes·KOH, pH 7.5) as described under *Preparation of MV*, except that these steps were performed at 25°C. Total protein obtained per dish was 3.7 ± 0.94 mg. The homogenate was centrifuged at 10,000 × *g* for 10 min at 4°C. The pellet contained 28–30% of the protein. The postnuclear supernatant (PNS), which contained 70–72% of the protein, was layered on a sucrose step gradient (20%, 30%, and 38% sucrose in 10 mM Hepes·KOH, pH 7.5) and centrifuged at 40,000 rpm in a Beckman SW-41 rotor for 2 hr at 4°C. Fractions 1–4 contained 5–7% of the protein, distributed as follows: 21 ± 5%, 31 ± 6%, 16 ± 5%, and 26 ± 7% in fractions 1–4, respectively. These fractions were collected, diluted 30-fold in buffer A, centrifuged at 100,000 × *g* for 2 hr at 4°C, and resuspended in buffer A.

α-Mannosidase II Activity. α-Mannosidase II was assayed by a modification of a reported procedure (23). A 50-μl aliquot (50 μg) from each sample was diluted with 520 μl of phosphate-buffered saline (137 mM NaCl/2.4 mM KCl/10.4 mM Na_2HPO_4/1.8 mM KH_2PO_4, pH 7.2) containing 25 μg of 4-methylumbelliferyl β-D-mannopyranoside (Sigma), 0.1% Triton X-100, and 2.5 mg of bovine serum albumin. Reaction mixtures were incubated at 25°C for 4 hr and terminated by the addition of 1.43 ml of 0.5 M glycine/0.5 M Na_2CO_3. Hydrolysis of 4-methylumbelliferyl β-D-mannopyranoside was detected by the fluorescence emitted by the samples at 480 nm (excitation at 355 nm).

RESULTS

MV prepared from FRTL-5 cells exhibited Na^+-dependent I^- accumulation (Fig. 1). Virtually no I^- translocation was detected when Na^+ was replaced with choline in the medium. I^- accumulation was inhibited by perchlorate, a competitive inhibitor of thyroidal I^- transport (1–3). Minimal intravesicular levels of I^- were observed after prolonged incubation (100 min) of the MV in the presence or absence of Na^+, due to the gradual dissipation of the Na^+ gradient and the consequent halt of active Na^+–I^- symport activity (Fig. 1). No active I^- accumulation was observed when MV were treated with the detergent Triton X-100 or with hypoosmotic solution. As expected, MV prepared from various control non-I^--transporting cell lines exhibited no Na^+–I^- symport activity (Fig. 1).

Surprisingly, an ≈2-fold increase in Na^+–I^- symport activity was consistently observed when MV from FRTL-5 cells were prepared under conditions that favor proteolysis (i.e., at 25°C in the absence of protease inhibitors; Fig. 2), as compared to the activity measured when the standard procedure (at 4°C in the presence of protease inhibitors) was used. The effects of temperature and inhibitors appeared to be additive, since MV prepared at 25°C in the presence of protease inhibitors exhibited Na^+–I^- symport activity at an intermediate level with respect to the other two conditions (Fig. 2). MV prepared from rat thyroids under the mentioned proteolysis-favorable conditions also exhibited an ≈2-fold increase in Na^+–I^- symport activity with respect to control rat thyroid MV (data not shown). Therefore, the enhancement of Na^+–I^- symport activity by proteolysis is evident in MV prepared both from cells in culture and from native tissue.

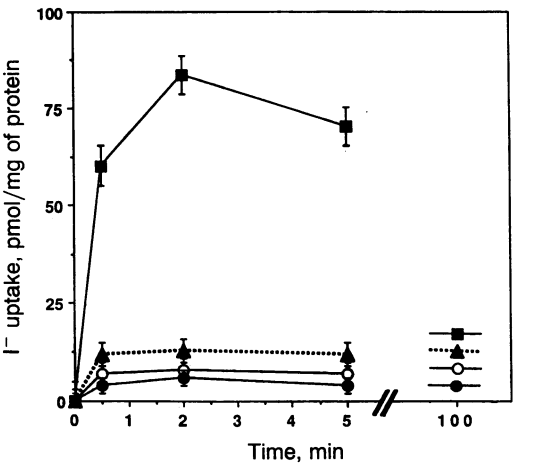

FIG. 1. I^- uptake in MV prepared from FRTL-5 cells. MV from TSH(+) FRTL-5 cells were prepared at 4°C in the presence of protease inhibitors (*Experimental Procedures*). Aliquots (10 μl; 50 μg of protein) were assayed for $^{125}I^-$ uptake at the indicated times by incubation at 25°C in an equal volume (10 μl) of 20 μM $Na^{125}I$ (1.1 Ci/mmol)/1 mM $MgCl_2$/10 mM Hepes·KOH, pH 7.5)/2 mM methimazole with 200 mM NaCl (■), or 200 mM NaCl plus 30 μM $NaClO_4$ (●), or 200 mM choline chloride (○). ▲, Na^+-dependent I^- uptake in MV from 3T3-L1 adipocytes and fibroblasts, MCF-7 cells and COMMA 1-D cells and in MV from FRTL-5 cells assayed in the presence of 1% (vol/vol) Triton X-100 or quenched with water. Reactions were terminated at the times shown by the addition of 4 ml of ice-cold quenching solution (250 mM KCl/1 mM Tris·HCl, pH 7.5)/1 mM methimazole, followed by rapid filtration through wet nitrocellulose filters (0.45 μm). Filters were washed twice with an additional 4 ml of quenching solution. Radioactivity retained by MV was determined by a γ counter. Error bars indicate SD of triplicate determinations. Values at saturation between experiments ranged from 70 to 120 pmol of I^- per mg of protein (n = 4 experiments).

I^- accumulation observed in MV prepared from FRTL-5 cells under conditions that promote proteolysis was ≈200 pmol/mg of protein (Fig. 2). This level of accumulation

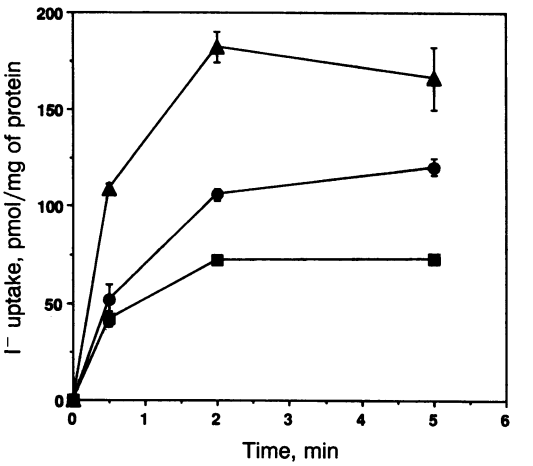

FIG. 2. I^- uptake in MV prepared from FRTL-5 cells under conditions that favor proteolysis. I^- uptake was measured in MV prepared from TSH(+) FRTL-5 cells at 25°C in the presence (●) or absence (▲) of protease inhibitors or at 4°C in the presence of protease inhibitors (■). Error bars represent SD of duplicate determinations. Values at saturation between experiments in MV prepared at 25°C in the absence of protease inhibitors ranged from 160 to 280 pmol of I^- per mg of protein (n = 6 experiments). Basal levels of associated I^- due to binding and/or low levels of passive diffusion (i.e., not mediated by the Na^+–I^- symporter) were obtained by using choline in place of Na^+. These basal values, which in all cases were <10% of values obtained in the presence of Na^+, were subtracted so that data reflect only Na^+-dependent values.

generated an ≈20-fold intravesicular I^- concentration gradient with respect to the extravesicular medium, as calculated from a determined intravesicular volume of 1.02 ± 0.12 μl/mg of protein. This value is consistent with I^- accumulation gradients reported in the thyroid gland *in vivo* (20- to 40-fold; refs. 1–3).

Na^+–I^- symport activity was measured in TSH(+) and TSH(−) FRTL-5 cells (Fig. 3 *Inset*). Activity was also assayed in MV prepared from TSH(+) cells and from cells that had been deprived of TSH for >7 days (arrow in Fig. 3 *Inset*). While Na^+–I^- symport activity decreased and eventually stopped in TSH(−) cells at the end of the 7-day period (Fig. 3 *Inset*) as previously reported (7), Na^+–I^- symport activity was unexpectedly observed in MV prepared from these non-I^--transporting cells (Fig. 3). The levels of transport in these MV were nearly identical to those recorded in MV from TSH(+) cells (i.e., leading to an ≈20-fold intravesicular I^- concentration gradient with respect to the extravesicular medium). Moreover, Na^+–I^- symport activity detected in MV from TSH(−) cells displayed characteristics identical to those found in MV from TSH(+) cells; i.e., I^- transport was Na^+-dependent and was competitively inhibited by perchlorate (Fig. 3). In addition, minimal intravesicular I^- was observed after prolonged incubation of TSH(−) MV in the presence or absence of Na^+, and no symport activity was detected when TSH(−) MV were in the presence of Triton X-100 or hypoosmotic solution (data not shown). Hence, components that are necessary and sufficient to mediate Na^+–I^- symport activity appear to remain present in FRTL-5 cells that, as a result of TSH deprivation, have ceased to exhibit Na^+–I^- symport activity. Further, when TSH(−) MV were prepared under the above-described conditions that promote proteolysis, an ≈2-fold increase in Na^+–I^- symport activity with respect to control MV (prepared under conditions not favoring proteolysis) was also observed (data not shown).

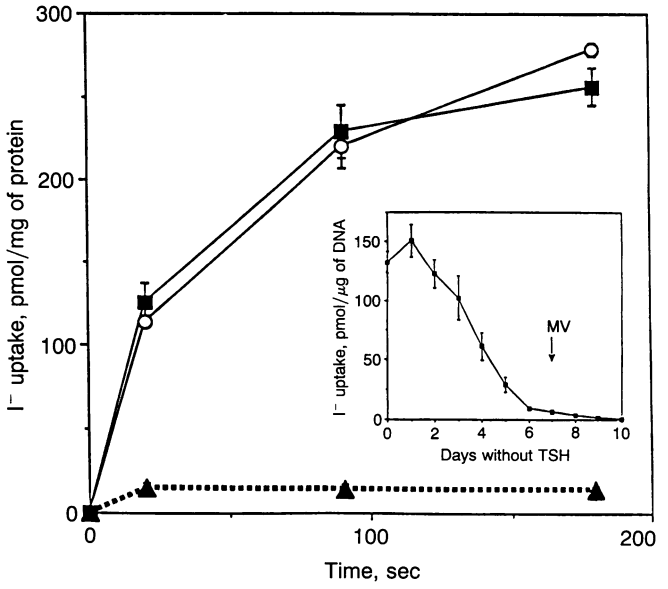

FIG. 3. I^- uptake in FRTL-5 cells and MV. MV were prepared at 25°C in the absence of protease inhibitors from FRTL-5 cells maintained in the presence (■) or absence (7 days) (○) of TSH and were assayed for I^- transport activity as for Fig. 1. Solid lines represent assays performed in the presence of Na^+, and the dotted line represents assays performed in MV from TSH(−) cells in the presence of Na^+ and 30 μM $NaClO_4$. (*Inset*) FRTL-5 cells were maintained in the presence of TSH (day 0) or in the absence of TSH for the indicated number of days. I^- uptake was initiated by adding to each well 500 μl of Hanks' balanced salts solution (HBSS)/10 mM Hepes, pH 7.0/10 μM $Na^{125}I$ (50 Ci/mol). Cells were incubated at 37°C for 30 min. Reactions were terminated by aspirating the radioactive solution and washing three times with HBSS/10 mM Hepes, pH 7.0. Accumulated $^{125}I^-$ was determined by permeabilizing the cells with cold ethanol for 20 min and quantitating the released radioisotope in a γ counter. DNA was determined by the diphenylamine method (24) on each well. Basal values in FRTL-5 cells were <5% of values obtained in the presence of Na^+ and were subtracted so that data reflect only Na^+-dependent values. Error bars indicate SD of triplicate determinations.

According to the redistribution hypothesis (Fig. 4 *a–c*), Na^+–I^- symporter molecules would reside in intracellular organelles in the absence of TSH (Fig. 4*a*). Readdition of TSH would recruit the symporter to the PM (Fig. 4*b*). A similar mechanism involving the redistribution of glucose

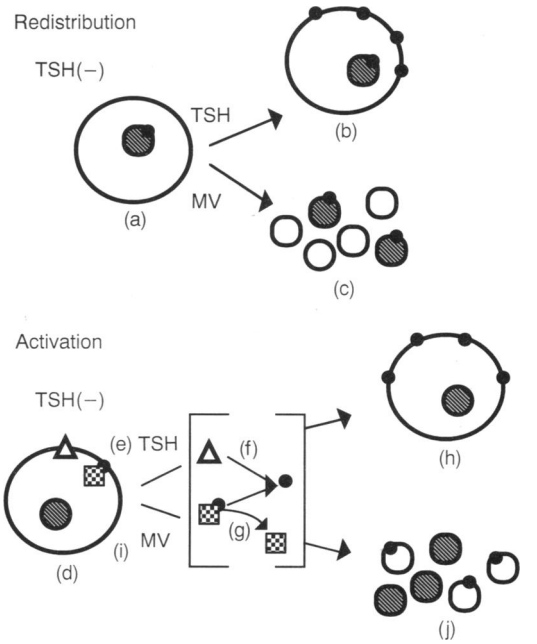

FIG. 4. (*Upper*) Redistribution hypothesis. *a* represents FRTL-5 cells maintained in the absence of TSH (7 days) with the Na^+–I^- symporter (filled circle) in intracellular structures (hatched circle); upon addition of TSH, symporter molecules are recruited to the PM (*b*). MV preparation generates PM vesicles (open circles) and intracellular organelle vesicles (hatched circles) (*c*). (*Lower*) Activation hypothesis. *d* represents FRTL-5 cells maintained in the absence of TSH (7 days) with Na^+–I^- symporter that has not been activated (open triangle), or with an inhibitor (checkered square) bound to the Na^+–I^- symporter (filled circle); an intracellular membrane structure is also shown (hatched circle); upon addition of TSH (*e*) the symporter is activated (*f*) or the inhibitor is removed (*g*), leading to active symporter molecules in the PM (*h*). MV preparation mimics the effect of TSH (*f* or *g*), leading to active symporter molecules in PM vesicles (*j*).

carrier molecules has been documented for the stimulation of glucose uptake by insulin. This redistribution, however, is detected within 10 min after addition of insulin (25), whereas maximal stimulation of Na^+–I^- symport activity by TSH is observed after 60 hr (8). Preparation of MV from TSH(−) cells would expose putative organelle Na^+–I^- symporter molecules to the external milieu (Fig. 4*c*). Thus, the model predicts that transport activity would be associated with intracellular membranes.

In the activation model (Fig. 4 *d–j*), the Na^+–I^- symporter would constitutively reside in the PM in a nonfunctional state (Fig. 4*d*) and would require TSH for activation (Fig. 4*e*). Possible mechanisms of activation include posttranslational modifications (e.g., phosphorylation, proteolytic processing) or interaction of a noncovalent activator with the Na^+–I^- symporter. TSH could also promote the removal of a posttranslational modifying group or of a noncovalent inhibitor (Fig. 4*g*). Therefore, addition of TSH would lead to the presence of active Na^+–I^- symporter molecules in the PM (Fig. 4*h*). TSH could also simultaneously increase biosynthesis of the symporter and/or other proteins that mediate the activation process. Preparation of MV from TSH(−) cells would mimic TSH activation of the symporter by either exposing the symporter to an activator (Fig. 4*f*) or removing an inhibitor (Fig. 4*g*). On the other hand, increased protein biosynthesis would clearly play no role in the Na^+–I^- symport activity in MV from TSH(−) cells. The activation model predicts that the bulk of transport activity detected in MV from TSH(−) cells would be associated with PM vesicles (Fig. 4*j*).

To determine whether intracellular membrane sources of Na^+–I^- symporter exist, and to test the proposed hypotheses, symport activity was measured in subcellular membrane fractions from TSH(+) and TSH(−) cells. To ascertain the relative presence of the various membrane sources in the fractions obtained (F1–F4), immunoblot analyses were performed with antibodies against organelle-specific markers: anti-α subunit of the Na^+/K^+-ATPase for PM (26), anti-ribophorin for endoplasmic reticulum (ER) (27), and anti-TGN 38/41 for Golgi membranes (28). A representative experiment performed with fractions from TSH(−) cells is shown in Fig. 5 *A–C*. Indistinguishable results were obtained in fractions from TSH(+) cells (data not shown). Analysis with anti-Na^+/K^+-ATPase α subunit (Fig. 5*A*) revealed that the corresponding ≈105-kDa immunoreactive polypeptide was present in the total homogenate (TH) and nuclear pellet (NP), it clearly predominated in F2 and F3, and was detected to a lesser extent in F4. No immunoreactivity was observed in F1. Analysis with anti-ribophorin (Fig. 5*B*) revealed immunoreactivity with an ≈67-kDa polypeptide in the TH and NP, to a modest degree in F2, noticeably higher in F3 and still higher in F4. Similarly, no immunoreactivity was observed in F1. In short, F2 and F3 contained most of the PM, and F3 and F4 contained most of the ER. While both PM and ER were detected to some extent in F2–F4, neither PM nor ER was present in F1.

In contrast, immunoreactivity of the Golgi-specific marker TGN-38/41 with its corresponding antibody was observed exclusively in F1 (data not shown). This observation suggests that F1 is highly enriched in Golgi membranes. The relative presence of Golgi membranes in the obtained fractions was also assessed by measuring the activity of α-mannosidase II, a Golgi-specific marker enzyme. α-Mannosidase II activity was predominantly associated with F1 (Fig. 5*C*), thereby confirming the immunoblot findings. Na^+–I^- symport activity was mostly associated with F2 and F3 (Fig. 5*D*)—i.e., the fractions most enriched in PM. Modest Na^+–I^- symport activity was also observed in F4, which was shown by immunoblot analysis to be rich in ER and to contain only a small amount of PM. Virtually no Na^+–I^- symport activity was measured in the Golgi membrane-containing F1. If the mechanism of stimulation of Na^+–I^- symport by TSH involved recruitment of intracellular Na^+–I^- symporter molecules, the ratio of symport activity in intracellular membranes to PM would be greater in TSH(−) than in TSH(+) cells. This was not observed, as the distribution of Na^+–I^- symport activity was indistinguishable in fractions from TSH(+) and TSH(−) cells (compare solid and hatched bars in Fig. 5*D*). These results favor the activation model over the redistribution model and suggest that the Na^+–I^- symporter may constitutively reside in the PM.

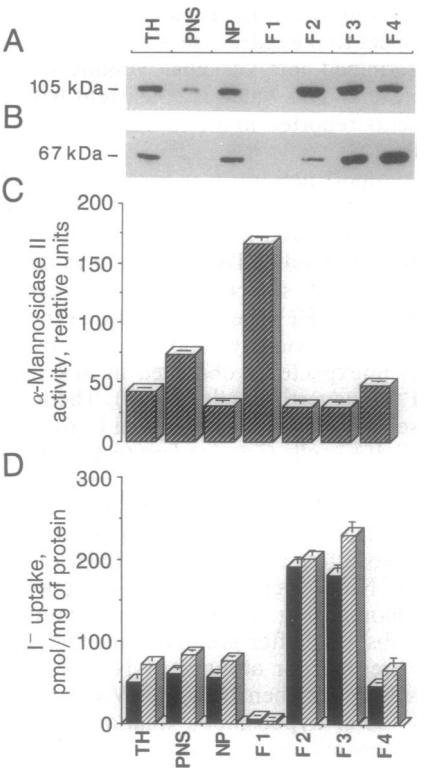

FIG. 5. Analysis of subcellular fractions from TSH(−) FRTL-5 cells. Cells were fractionated as described under *Experimental Procedures*. Fractions were collected as follows: F1, material above 20% sucrose; F2, material at 20–30% sucrose; F3, material just above 38% sucrose; F4, everything below 38% sucrose, including the pellet. In addition, the following aliquots were saved: TH, total homogenate; PNS, postnuclear supernatant; NP, nuclear pellet. For immunoblots, polypeptides were electrophoresed, electrotransferred, and processed as described under *Experimental Procedures*. (*A*) Fifteen micrograms of protein (heated at 37°C for 30 min) was loaded onto each lane and anti-Na^+/K^+-ATPase α subunit (Upstate Biotechnology) was diluted 1:500. (*B*) Ten micrograms of protein (boiled for 5 min) was loaded onto each lane and anti-ribophorin (gift from G. Kreibich, New York University) was diluted 1:200. Quantitation was performed by densitometry analysis (Molecular Dynamics) of multiple exposures of ECL (Amersham)-visualized bands. Enrichment factors are given relative to presence of specific marker in PNS: α subunit of Na^+/K^+-ATPase (F2 and F3), 5- to 7-fold; ribophorin (F4), 7- to 9-fold; n = 8 experiments. (*C*) Golgi α-mannosidase II activity was measured in assays using 50 μg of protein. Error bars indicate SD of duplicate determinations. Enrichment of α-mannosidase II (F1) over PNS ranged from 3-fold to 6-fold (n = 6). (*D*) I^- uptake was performed exactly as in Fig. 1. Error bars indicate SD of duplicate determinations (2-min time point) of fractions from TSH(−) cells (hatched bars) or from TSH(+) cells (solid bars) (n = 8).

DISCUSSION

As a system to further characterize the activity of the Na^+–I^- symporter, MV were prepared from FRTL-5 cells. Unlike previous studies in thyroid MV (9–11), in which vesicles were derived from intact glands, the present experiments were performed with MV prepared from a line of thyroid cells in culture. Highly effective Na^+–I^- symport activity was mea-

sured in MV prepared from FRTL-5 cells (Fig. 2). The ≈20-fold I^- concentration gradient generated by these MV was comparable to I^- concentration gradients previously reported: ≈30-fold in FRTL-5 cells (7) and 20- to 40-fold in the thyroid gland *in vivo* (1–3).

Proteolysis has been reported to play a role in the activation of some transporters, including the plasmalemma Na^+/Ca^{2+} exchanger (29) and the erythrocyte PM Ca^{2+}-ATPase (30). The observation that Na^+–I^- symport activity in vesicles from the TSH(+) or TSH(−) FRTL-5 cells or from rat thyroid glands is enhanced when conditions for MV preparation favor proteolysis (Fig. 2) shows that proteolysis (directly or indirectly) activates the Na^+–I^- symporter and that a proteolytic event might be at least one step in the mechanism by which TSH stimulates Na^+–I^- symport activity.

The central finding of this report is that pronounced Na^+–I^- symport activity was unexpectedly detected in MV from TSH(−) FRTL-5 cells. Transport observed in these MV was of the same magnitude and pharmacological characteristics as in MV from TSH(+) cells (Fig. 3). Moreover, the K_m values for I^- were nearly identical in the two groups of MV [≈20 μM for TSH(+); ≈18 μM for TSH(−); J. Xiong and N.C., unpublished results]. These K_m values for I^- are in complete agreement with those reported in the literature for FRTL-5 cells, 5–49 μM (7, 31, 32), as well as with those in thyroid-derived preparations from various species, 5–40 μM (1–3, 9).

These observations demonstrate that vesicular Na^+–I^- symport activity is catalyzed by components present in both TSH(+) cells and cells that have been maintained in the absence of TSH for >7 days, even though the latter exhibit virtually no Na^+–I^- symport activity (Fig. 3 *Inset*). Therefore, the absence of Na^+–I^- symport activity detected in TSH(−) cells cannot result solely from a decrease in the biosynthesis of either the symporter or a putative activating factor. A possible (though unlikely) mechanism involving the Na^+/K^+-ATPase could explain the mentioned finding. However, the presence or absence of TSH had no apparent effect on the activity of the Na^+/K^+-ATPase. Therefore, the redistribution and activation models were proposed (Fig. 4). Since subcellular fractionation studies of TSH(+) and TSH(−) cells revealed that vesicular Na^+–I^- symport activity was associated with fractions predominantly containing PM in both cases, rather than membranes from intracellular organelles, it is possible that the Na^+–I^- symporter constitutively resides in the PM and requires TSH for activation. Studies using an anti-Na^+–I^- symporter antibody would strengthen the mentioned conclusion, but to our knowledge no such antibodies are presently available. Still, similar findings in other systems have recently come to light. Inactive glucose transporters have been reported to reside in the PM of rat adipocytes (33) and 3T3 L-1 adipocytes (34). In the present report the ratio of I^- accumulation in intracellular membranes to PM was not higher in the fractions from TSH(−) cells with respect to the fractions from TSH(+) cells (Fig. 5*D*). Thus, all the above results support the model of TSH activation of Na^+–I^- symporter molecules that constitutively reside in the PM of thyroid cells, in addition to the previously known effect of the hormone to bring about *de novo* protein biosynthesis of relevant molecules. While more studies are clearly needed to elucidate the molecular basis of the stimulation of I^- uptake by TSH, the unexpected Na^+–I^- symport activity observed in MV from TSH(−) FRTL-5 cells offers another perspective on the nature of TSH regulatory mechanisms.

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