MCT1 and its accessory protein CD147 are differentially regulated by TSH in rat thyroid cells

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Fanelli, Albertina, Evelyn F. Grollman, Dian Wang, and Nancy J. Philp. MCT1 and its accessory protein CD147 are differentially regulated by TSH in rat thyroid cells. Am J Physiol Endocrinol Metab 285: E1223–E1229, 2003; 10.1152/ajpendo.00172.2003.—In thyroid cells, basal and TSH-stimulated glycolysis is associated with lactic acid efflux. In this report, we address whether monocarboxylate transporters (MCTs) are present in thyroid tissue for exporting excess lactic acid generated by aerobic glycolysis. Using immunostaining techniques, we show that MCT4 localizes with its accessory protein CD147 in the basolateral membrane of rat thyroid follicular cells. In cultured rat thyroid (FRTL-5) cells, MCT1 rather than MCT4 is expressed. CD147 colocalizes and coimmunoprecipitates with MCT1. TSH upregulates MCT1/CD147 expression as a function of time through a cAMP-dependent mechanism as forskolin reproduces the effect of TSH. TSH enhances protein expression of both MCT1 and CD147 in FRTL-5 cells. Whereas MCT1 protein expression is controlled at the level of transcription, CD147 protein expression is regulated by a posttranscriptional mechanism. Results of these studies suggest that hormone stimulation of lactate transport is mediated by regulating MCT1 transcription.

IN THE THYROID GLAND, the primary metabolic fuels are glucose and free fatty acids (1, 7, 8). Oxidative phosphorylation uses mainly free fatty acids as substrate and accounts for >80% of ATP production. Mitochondrial respiration is primarily regulated by the ADP supply, and the stimulatory effect of TSH and cAMP is secondary to enhanced cellular processes that consume ATP. Aerobic glycolysis accounts for 70% of the glucose metabolized by the thyroid cell and supplies almost 20% of the cellular ATP (reviewed in Ref. 8). It has been suggested that glycolytic ATP may be the preferred source of energy for plasma membrane functions such as iodide transport and colloid endocytosis (4, 9, 27).

Early on, it was suggested that glycolytic ATP in thyroid could be important for the energy requirements of Na⁺-K⁺-ATPase and processes dependent on ATP. In studies of sheep thyroid slices, when respiration was inhibited and iodide transport blocked, addition of glucose partially restored iodide transport (27). In other studies, inhibition of glycolysis greatly inhibited colloid endocytosis, and glucose addition counteracted this effect (9). These studies suggest that aerobic glycolysis is important for membrane enzyme and transport activities. More recent studies in rat skeletal muscle show that there is a strong link between aerobic glycolysis and Na⁺-K⁺-ATPase activity (15). Diverse treatments, known to raise muscle intracellular Na⁺ concentration and increase Na⁺-K⁺-ATPase activity, stimulated aerobic glycolysis and lactate production. Whether a similar set of events operates in thyroid is unknown, but sodium influx is an early event following TSH stimulation in thyroid tissue (8).

TSH increases aerobic glycolysis in the thyroid gland by stimulating an increase in glucose uptake (7, 8, 14). Studies of TSH effects on glucose metabolism in the thyroid cell line FRTL-5 found enhanced glucose uptake associated with an increase in expression and translocation of the glucose transporter GLUT1 (13, 25). GLUT1 is not detected in normal human thyroid tissue (11) and whether another GLUT isoform is associated with TSH-stimulated glucose uptake in thyroid has yet to be demonstrated.

Glucose uptake in thyroid, both basal and TSH stimulated, is coupled with lactic acid efflux (7, 14, 20). Tissues that utilize aerobic glycolysis require the export of lactic acid for glycolysis to continue at a high rate and to avoid a drop in intracellular pH (12, 16). Transport of lactic acid across the plasma membrane is accomplished by proton-linked monocarboxylate transporters (MCTs). In mammals, nine distinct MCT isoforms have been cloned that differ in their tissue distribution and in their sequence homology, most notably in the COOH terminus (reviewed in Ref. 12). The structural and functional properties of MCT1-MCT4 have been studied extensively. They have a molecular mass of 40–50 kDa and are not glycosylated (12, 31). MCT1 is found in a majority of tissues, whereas MCT4 predominates in cells with high rates of aerobic glycolysis, for example, white muscle fibers, lymphocytes, and tumor cells (12).

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Recent studies show that, when MCT1, MCT3, or MCT4 (but not MCT2) is present in the plasma membrane, it is invariably found with at least one other protein, CD147 (17, 21). CD147 is a widely expressed membrane glycoprotein and a member of the immunoglobulin supergene family with NH2-linked glycans responsible for as much as 50% of the mass (19). From studies of a CD147-null mouse, one documented function of CD147 is trafficking of MCTs to the plasma membrane (21). Because there is no evidence of a disulfide linkage, this trafficking function probably involves a noncovalent steric association. Whether CD147 also affects transport activity of MCTs is unknown.

In this report, we examine MCT and CD147 expression in rat thyroid tissue and in FRTL-5 cells. FRTL-5 cells are a continuous line of differentiated thyroid cells that can be maintained in the absence of TSH but respond to the addition of TSH with growth and differentiated functions (10, 29). We document the expression of MCT4 and CD147 in rat thyroid tissue and show that TSH enhances by separate mechanisms the expression of MCT1 and CD147 in FRTL-5 cells.

MATERIALS AND METHODS

Cell culture. FRTL-5 cells (American Type Culture Collection no. CRL 8305, F1 clone), a TSH-responsive rat thyroid cell line, were maintained in Coon’s modified Ham’s F-12 medium (Sigma) supplemented with 5% calf serum (Invitrogen Life Technologies), 1 × 10^{-4} M nonessential amino acids (Biofluids), and a mixture of hormones (6H) consisting of 1 × 10^{-10} M bovine TSH, 10 μg/ml insulin, 0.4 ng/ml cortisol, 5 μg/ml transferrin, 10 ng/ml somatostatin, and 2 ng/ml glycyl-l-histidyl-l-lysyl acetate. Cells were grown in a humidified atmosphere at 37°C. Culture medium was changed every 2–3 days, and cells were passaged weekly using 0.05% trypsin-0.025% versene (Biofluids). In some experiments, cells were maintained for 1 wk in medium supplemented with hormone mixture free of TSH (5H) and then incubated for the time indicated with 6H or 5H medium or 5H medium containing 10 μM forskolin (Sigma).

Rat thyroid tissue. Animal experimentation conformed to the Helsinki convention for animal care and use. Male Sprague-Dawley rats (125–150 g) were purchased from Charles River (Wilmington, MA) and were euthanized with pentobarbital sodium (150 mg/kg body wt).

Antibodies. Anti-peptide antibodies were raised in rabbits against the synthetic oligopeptide corresponding to the carboxyl-terminal amino acids of MCT1, MCT2, MCT3, and MCT4, as previously described (22, 23). Antibody specificity was confirmed by immunostaining in the presence and absence of excess peptide used for immunization. Anti-CD147 antibody (RET-PE2) was the generous gift of Colin J. Barnstable (Yale University School of Medicine, New Haven, CT). GLUT1 antibody was kindly provided by Ian Simpson (Pennsylvania State University, Hershey, PA).

Immunofluorescence microscopy. Cryosections (6–10 μm) were cut and mounted on Superfrost/Plus glass microslide slides (Fisher Scientific), air dried at room temperature, and stored at −80°C.

FRTL-5 cells (10^5 cells/well), plated on Permanox chamber slides (Nalge Nunc) were grown for 1 wk in 5H medium and then in 5H or 6H medium for an additional 72 h. After being rinsed three times in PBS, cells were fixed for 1 h in 3.5% formaldehyde in PBS, washed in PBS, then permeabilized in cold methanol for 5 min, and then rinsed in PBS before blocking.

FRTL-5 cells and rat thyroid sections were incubated for 1 h in blocking solution (5% BSA-0.1% Tween-20 in PBS) and then for 1 h in anti-MCT1, -MCT2, -MCT3, -MCT4, or -CD147 antibody diluted 1:200 in 1% BSA-0.1% Tween-20 in PBS and then washed four times with PBS-0.1% Tween-20. Primary antibodies were detected using carboxymethylindocyanine-coupled secondary antibody (1:500; Jackson Immunoresearch Laboratories, West Grove, PA) diluted in 1% BSA-0.1% Tween-20 in PBS. Sections were examined with an Nikon Microphot FX microscope equipped with an Optronics digital camera and Bioquant imaging software.

Western blotting. FRTL-5 cells were extracted with ice-cold lysis buffer [1% Triton X-100, 10 mM imidazole, 100 mM KC1, 1 mM EDTA, 5 mM MgCl2, and protease inhibitors (Complete Mini; Roche Applied Science)] for 30 min and then centrifuged at 14,000 g at 4°C for 15 min. The protein concentration of the cleared lysates was determined using BCA Reagent (Pierce). Lysates, diluted 1:1 in 2× SDS sample buffer (10 μg protein/well), were separated on 4–12% SDS-polyacrylamide gels and then transferred electrophoretically to Immobilon-P membranes (Millipore). Membranes were incubated for 1 h at room temperature in blocking buffer (5% BSA-20 mM Tris-HCl, pH 7.5, 137 mM NaCl) followed by a 1-h incubation with primary antibodies diluted 1:2,000 and secondary antibody diluted 1:3,000 in dilution buffer (1% BSA-20 mM Tris-HCl, pH 7.5, 137 mM NaCl). Reactive bands were visualized with an enhanced chemiluminescence detection system (ECL; Amersham Pharmacia Biotech).

Biotinylation of cell surface proteins. FRTL-5 cells, grown to 80–90% confluence in 100-mm tissue culture dishes, were washed twice with ice-cold Hanks’ balanced salt solution (HBSS) and then incubated in HBSS containing 0.5 mg/ml EZ Link Sulfo-NHS-LC-Biotin (Pierce) for 30 min on ice. The reaction was quenched by washing the plates three times with 10 mM Tris-HCl (pH 7.4) and 154 mM NaCl and twice with ice-cold HBSS. Cells were extracted in 1 ml of ice-cold lysis buffer containing protease inhibitors. Lysates were transferred to 1.5-ml microfuge tubes, incubated on ice for 20 min, and cleared by centrifugation at 14,500 rpm. An aliquot of the cleared lysates was removed for protein determination, and another aliquot was diluted 1:1 with 2× sample buffer for analysis of biotinylated proteins by SDS-PAGE and blotting with avidin-peroxidase (Sigma). The remaining lysates were used for immunoprecipitation with anti-CD147 antibody. Lysates were incubated with 20 μl of protein G-agarose (Sigma) for 1 h with rotation at 4°C and centrifuged. The supernatants were transferred to new tubes and incubated with 10 μl of undiluted anti-CD147 antibody for 2–18 h with end-over-end rotation at 4°C. Protein G-agarose (25 μl) was added to the sample and incubated for an additional 2 h. The sample was centrifuged at 1,500 rpm for 5 min. An aliquot of the supernatant was removed and diluted with 2× sample buffer for analysis of unbound protein. The protein G-agarose pellet was washed five times with 250 μl of lysis buffer, and immunoprecipitated proteins were then eluted from the agarose with 2× SDS sample buffer. Immunoprecipitated proteins were separated by SDS-PAGE and transferred to Immobilon-P membranes as previously described (23). Blots
were probed with avidin-peroxidase or anti-MCT1 antibody followed by a horseradish peroxidase-coupled donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories) and detected using ECL (Amersham Pharmacia Biotech).

RT-PCR analysis. Total RNA was extracted from FRTL-5 cells using TRIzol (Invitrogen Life Technologies) following the manufacturer's instructions. Expression of MCT mRNAs in FRTL-5 cells was determined using RT-PCR with isoform-specific primers. First-strand cDNAs were prepared using 1.5 μg of total RNA and 3'-RACE Adaptor Primer (3AP, 5'-ggc cac gcc tgg act act ttt ttt ttt t-3'; Invitrogen Life Technologies). MCT1 was amplified using the following primer sets: MCT1 (forward primer: 5'-gga ggt ctt ggg ctt gct-3'; reverse primer: 5'-gtt gcc cta gcc cac agc act-3'; cycling parameters: 94°C, 30 s; 62°C, 30 s; 72°C, 60 s; 25 cycles) and CD147 (forward primer: 5'-gtt gcc cta gcc cct ggt ttc a-3'; reverse primer: 5'-ggt cac caa gcc cac agc act-3'; cycling parameters: 94°C, 30 s; 62°C, 30 s; 72°C, 60 s; 20 cycles). PCR products were separated on a 1.0% agarose gel. PCR fragments were gel-purified using a QIAEX II (Qiagen) extraction kit and sequenced at the Nucleic Acid Facility in the Kimmel Cancer Institute at Thomas Jefferson University.

Lactic acid export. FRTL-5 cells were grown to confluence in six-well dishes for 1 wk in the absence of TSH and then with or without TSH for an additional 3 days. After removal of the media, the cells were washed twice with glucose-free HBSS. To assay lactate release, 2 ml of HBSS with or without glucose were added to each well. Incubation was carried out at 37°C with aliquots (25 μl) being removed at 0.5, 1, and 2 h. Lactic acid was measured using a lactic acid dehydrogenase-based kit (826-UV; Sigma). Protein was determined using the BCA protein assay (Pierce).
Lactic acid export in FRTL-5 cells. Lactic acid export in FRTL-5 cells was measured by sampling the incubation medium over a 2-h period. The export was linear during this time, and the rate of export is shown in Fig. 3. Lactic acid export was 12 times higher in cells grown with TSH compared with cells grown without TSH. There was no release of lactic acid when glucose was not present in the incubation medium. Lactic acid release from cells grown with TSH was reduced 78% when the MCT-specific inhibitor α-cyano-4-hydroxycinnamate (24) was added.

Effect of TSH on GLUT1, MCT1, CD147, and actin protein expression in FRTL-5 cells. The expression of MCT1, CD147, and GLUT1 proteins was examined in FRTL-5 cells grown in the absence or presence of TSH. Immunofluorescence studies demonstrated that TSH was required for expression of MCT1 and its accessory protein CD147. This observation was supported by Western blot analysis of FRTL-5 cell lysates prepared from cells depleted of hormone for 1 wk and then grown in the presence of TSH for 72 h (Fig. 4). TSH increased MCT1 and CD147 protein levels, as shown in Fig. 4, middle. In Fig. 4, top, the blot was probed with anti-GLUT1 antibody. The increase in GLUT1 protein seen in cells grown with TSH confirms published reports (13, 25). The amount of actin was similar in lysates of FRTL-5 cells grown with or without TSH (Fig. 4, bottom).

Coprecipitation of CD147 with MCT1 in FRTL-5 cell lysates. Cell surface biotinylation followed by immunoprecipitation was used to analyze CD147 expression in the plasma membrane of FRTL-5 cells grown in the presence or absence of TSH (Fig. 5). An anti-CD147 monoclonal antibody was used to immunoprecipitate CD147 from the detergent-soluble lysates of biotinylated cells. In CD147 immunoprecipitation blots probed with avidin-horseradish peroxidase (HRP), a broad band migrating between the 30- and 45-kDa markers

Fig. 3. Lactic acid export measured in FRTL-5 cells. FRTL-5 cells were grown to confluence in 6-well plates with (+) or without (−) TSH, and lactic acid content of the incubation medium was measured as detailed in MATERIALS AND METHODS. Incubation medium was Hanks’ balanced salt solution with (+) or without (−) glucose (GLC). In some wells, α-cyano-4-hydroxycinnamate (CHC) was added at a final concentration of 1 mM. Values shown are means ± SD of 3 independent experiments.

Fig. 4. Western blot analysis of the effect of TSH on GLUT1, MCT1, CD147, and actin expression in FRTL-5 cells. FRTL-5 cells were grown in the absence of TSH for 1 wk and then with or without 1 × 10−10 M TSH for 72 h. Detergent-soluble lysates were prepared from cells, and proteins (15 µg/lane) were separated on 4–12% gradient SDS gels and Western blotted. Quadruplicate blots were probed with antibodies to GLUT1, MCT1, CD147, and actin. Blots shown are representative of 3 independent experiments.

Fig. 5. Expression of CD147 in plasma membrane of FRTL-5 cells. FRTL-5 cells were grown with or without TSH, cell surface proteins were biotinylated, and CD147 was immunoprecipitated (IP) from detergent-soluble cell lysates with an anti-CD147 antibody monoclonal antibody. Precipitated proteins were separated on 4–12% gradient SDS gels and transferred to Immobilon-P membranes. A: immunoprecipitated CD147 protein present on the cell surface was detected using avidin-alkaline phosphatase and enhanced chemiluminescence, as detailed in MATERIALS AND METHODS. B: a duplicate blot of CD147 immunoprecipitated proteins was probed with anti-MCT1 antibody, as described in MATERIALS AND METHODS. C: total biotinylated proteins present in the detergent-soluble cell lysates (15 µg/lane) were detected with avidin-horseradish peroxidase.
was detected in cells cultured with TSH, whereas a faint band was detected in cells grown without TSH (Fig. 5A). The broad CD147 band is consistent with the glycosylation heterogeneity of this protein reported elsewhere (19). A duplicate blot was probed with anti-MCT1 antibody (Fig. 5B). MCT1 was found to coimmunoprecipitate with CD147. Total cell lysates from biotinylated FRTL-5 cells were probed with avidin-HRP (Fig. 5C) and show the overall level of surface labeling was similar in FRTL-5 cells grown in the presence and absence of TSH, although the pattern of protein expression is distinct.

**Time-dependent effect of forskolin on MCT1 and CD147 expression in FRTL-5 cells.** Many, but not all, thyroid functions dependent on TSH stimulation are mediated by cAMP (28). These TSH effects can be reproduced by nonspecific positive modulators of the cAMP cascade, as exemplified by forskolin, a stimulator of adenylate cyclase. Addition of forskolin to TSH-deprived cells led to a time-dependent increase in both MCT1 and CD147 protein expression. As shown in the Western blot in Fig. 6, there was a progressive increase in the levels of MCT1 and CD147 from 24 to 72 h after forskolin addition to cells grown in the absence of TSH.

**Differences in control of MCT1 and CD147 RNA expression.** To determine whether increased levels of MCT1 and CD147 in FRTL-5 cells resulted from effects of TSH and forskolin on transcription, we determined mRNA levels of MCT1 and CD147 by means of RT-PCR, as described in MATERIALS AND METHODS. FRTL-5 cells were depleted of TSH in the growth medium for 1 wk, followed by the addition of TSH or forskolin for the times indicated (Fig. 7). Effects of forskolin and TSH on MCT1 mRNA expression levels are similar to effects on MCT1 protein levels (Fig. 7 vs. Fig. 6). Only trace amounts of MCT1 transcript are seen in cells grown without TSH, but the transcript is detected at 24 h and maintained up to 72 h after TSH or forskolin addition. CD147 mRNA levels are unaltered by TSH or forskolin (Fig. 7). This disparity between TSH regulation of CD147 and MCT1 suggests that TSH control of MCT1 expression is at the level of transcription whereas control of CD147 expression is mediated by a posttranscriptional mechanism.

**DISCUSSION**

In this report, we demonstrate that MCT1 and CD147 expression in FRTL-5 cells is hormone dependent. TSH upregulates MCT1 and CD147 protein expression via a cAMP-dependent mechanism, supported by the observation that forskolin reproduced the effect of TSH. Enhancement of MCT1/CD147 protein expression induced by TSH and forskolin was time dependent, being maximal between 60 and 72 h following addition to TSH-depleted cells. Leptin is the only hormone previously shown to influence MCT expression (5). By use of an intestinal cell line (Caco2-BBE), leptin was shown to enhance MCT1 expression, which was accompanied by an increase in butyrate uptake (5).

TSH regulation of MCT1 and CD147 expression in FRTL-5 cells depended on separate mechanisms. TSH regulated MCT1 expression by increasing MCT1 transcription, as MCT1 mRNA and protein levels were elevated concordantly. TSH also increased CD147 protein levels, but CD147 transcript levels did not respond to TSH or forskolin addition. CD147 mRNA was expressed in cells grown without TSH and was unchanged after TSH or forskolin addition. The effect of TSH could depend on a posttranscriptional mechanism; alternatively, the increase in CD147 translation could be secondary to a protein (possibly MCT1) induced by TSH. This separate regulation of subunit synthesis (MCT1 transporter and CD147 accessory protein) may be a more general phenomenon, as a similar disparity has been described with heterodimeric amino acid transporters (6).

MCT and its accessory protein CD147 colocalized in the plasma membrane of thyrocytes and FRTL-5 cells. MCT1 was coimmunoprecipitated with CD147 from FRTL-5 cell lysates, demonstrating that the two proteins are associated. Recent studies suggest that lactate transporters, like other transporters, comprise a single-pass transmembrane glycoprotein (CD147) and a multipass transporter (MCTs) (3, 6). Colocalization of CD147 with MCT4 in the thyroid membrane is consistent with its proposed role in the proper translocation.
and targeting of MCTs to the plasma membrane (17, 21, 30).

MCT1 was found in FRTL-5 cells, whereas only MCT4 was found in rat thyrocytes. Isoform switching of MCTs has also been described in the ARPE-19 cell line. These cells have MCT4 in the basolateral membrane rather than MCT3, which characterizes retinal pigment epithelium in situ (22). The fact that FRTL-5 cells express MCT1 rather than MCT4 may reflect a less differentiated phenotype or different metabolic demands of cultured cells. This latter explanation is supported by the appearance of GLUT1 expression in these cells (13, 25). Although there is little or no GLUT1 protein in normal thyroid, both GLUT1 and MCT1 proteins are expressed in FRTL-5 cells. Unlike FRTL-5 cells, the elevated GLUT1 expression seen with tumor cells is associated with a decrease in MCT expression (18).

The presence of MCT1 in FRTL-5 cells correlated with the measured amount of lactic acid exported from the cells. Lactic acid export was more than 10 times greater in cells grown with TSH compared with cells grown in the absence of TSH. The export of lactic acid depended on glucose in the incubation medium. There was no lactic acid export measured when cells were incubated in glucose-free medium. The low glucose uptake reported for FRTL-5 cells grown without TSH could have contributed to the reduced levels of lactic acid loss seen in these cells.

In rat thyroid tissue, MCT4 and its accessory protein CD147 localized to the basolateral membrane of thyrocytes; there was no MCT detected in the apical membrane. MCT4 is the isoform commonly associated with tissues that rely on high levels of aerobic glycolysis for ATP production. It is a low-affinity, high-capacity lactate transporter capable of exporting lactate across the plasma membrane. About 20% of thyroidal ATP is supplied by aerobic glycolysis, and both basal and TSH-stimulated glycolysis is associated with lactic acid efflux (7, 14, 20). MCT4 in the basal membrane of the thyrocyte is a likely candidate for exporting excess lactic acid generated by aerobic glycolysis.

CD147 mRNA is expressed constitutively in FRTL-5 cells. CD147 protein expression in the plasma membrane, however, requires that MCT1 protein be expressed. When MCT1 is not expressed and unavailable for assembly to CD147, CD147 protein is likely degraded. In cancer cells where MCT expression can be downregulated or modified, CD147 may be target to a secretory rather than a degradative pathway (2, 26). Dependence of CD147 protein production on MCT expression allows for a cell with more than one MCT isoform, regulating independently the amount of a common accessory protein.

In summary, TSH regulates MCT1 transcription in FRTL-5 cells through a cAMP-dependent pathway. MCT1 is necessary for CD147 protein expression leading to MCT/CD147 subunit assembly and targeting to the plasma membrane.

DISCLOSURES

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