Thyroid hormones modulate zinc transport activity of rat intestinal and renal brush-border membrane

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Prasad, Rajendra, Vivek Kumar, Rajinder Kamur, and Kiran Pal Singh. Thyroid hormones modulate zinc transport activity of rat intestinal and renal brush-border membrane. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E774–E782, 1999.—Thyroid hormone status influences the Zn2+ and metallothionein levels in intestine, liver, and kidney. To evaluate the impact of thyroid hormones on Zn2+ metabolism, Zn2+ uptake studies were carried out in intestinal and renal brush-border membrane vesicles (BBMV). Steady-state Zn2+ transport in intestinal and renal cortical BBMV was increased in hyperthyroid (Hyper-T) rats and decreased in the hypothyroid (Hypo-T) rats relative to euthyroid (Eu-T) rats. In both the intestinal and renal BBMV, Hyper-T rats showed a significant increase in maximal velocity compared with Eu-T and Hypo-T rats. Apparent Michaelis constant was unaltered in intestinal and renal BBMV prepared from the three groups. Fluorescence anisotropy of diphenyl hexatriene was decreased significantly in intestinal and renal brush-border membrane (BBM) isolated from Hyper-T rats compared with Hypo-T and Eu-T rats. A significant reduction in the microviscosity and transition temperature for Zn2+ uptake in intestinal and renal BBM from Hyper-T rats is in accordance with the increased fluidity of these BBMs. These findings suggest that the increased rate of Zn2+ transport in response to thyroid hormone status could be associated with either an increase in the number of Zn2+ transporters or an increase in the activity of the transporters due to alterations in the membrane fluidity. Thus the thyroid hormone-mediated change in membrane fluidity might play an important role in modulating Zn2+ transport activity of intestinal and renal BBM.

Zn2+ is essential in many biochemical processes and may have a relevant part in the control of both cell proliferation and cell loss (5, 50). Zn2+ is essential for enzymes involved in DNA synthesis and mitosis (54), a component of many transcription factors and proteins that control the cell cycle (12, 45), and can inhibit apoptosis (29). Zn2+ deficiency affects cell cycle progression (38). Zn2+ is indispensable for biological processes like development, growth, differentiation, and function of the endocrine and nervous system and is also important for the maintenance of membrane structure and function (50). Zn2+ and thyroid function are related in several ways. Thyroid dysfunction influences Zn2+ metabolism (18) and vice versa. Thyroid hormones have a significant role in controlling growth and functions of intestine and kidney (23, 49). Thyroid hormones regulate cell proliferation and stimulate epithelial cell production (49). Thyroid hormones also regulate expression of brush-border membrane (BBM) enzymes of intestine (8, 19, 21). Dramatic structural and functional alterations in intestine induced by Zn2+ deficiency have been shown to be repaired by thyroxine (T4) treatment (36). Thyroid hormones also regulate renal plasma flow, glomerular filtration rate (GFR), reabsorption of phosphate and Ca2+ (25), Na+/K+-ATPase activity (30), and Na+/P, and Na+/H+ exchange activities in renal BBM (13, 26). In secondary Zn2+ deficiency in humans, decreased triiodothyronine (T3) and T4 levels and unresponsiveness of thyroid gland to thyrotropic hormone have been reported (35). On the other hand, hyperthyroid rats showed a variable distribution of Zn2+ in different organs with decreased levels of Zn2+ in kidney (11).

Intestine, liver, and kidney are of particular importance in maintaining Zn2+ homeostasis. Intestine and kidney are the major target organs for various regulators of mineral metabolism. The intestinal and renal absorptive cells are polar in nature. The membrane exposed to lumen (BBM) is functionally and structurally distinct from the basolateral membrane, which is in contact with extracellular fluid. The BBM is the first barrier encountered by various solutes during absorption in intestine and kidney (52). Zn2+ transport systems in renal and intestinal BBM have been well characterized (39, 41, 48). Zn2+ transport by brush-border membrane vesicles (BBMV) is a carrier-mediated, temperature-dependent, and saturable process. However, no information is available about the influence of putative regulators of Zn2+ metabolism at important steps such as absorption, reabsorption, and storage in intestine and kidney. The interaction between thyroid hormones and Zn2+, which are both required for growth and differentiation, prompted us to study the effect of thyroid hormone status on Zn2+ metabolism, especially with regard to the regulation of transport across the BBM in the intestine and kidney of rats.

MATERIALS AND METHODS

Chemicals. 65ZnCl2 (483 mCi/g Zn2+), d-[U-14C]glucose (292 mCi/mmol), and 110mAg+ (10 GBq/g Ag+) were purchased from Bhabha Atomic Research Center (Trombay, Mumbai, India). Thyroid powder (3 times recrystallized) was obtained from IFCI Clone Systems [Casselchio Di Reno(Bo)]. Ionophore A-23187, HEPES, EGTA, and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical (St. Louis, MO). Diphenyl hexatriene (DPH) was procured from Molecular Probes (Eugene, OR). Millipore
filters (pore size 0.45 µm) were obtained from Millipore (Bedford, MA). Glucose oxidase peroxidase kit was procured from Boehringer Knoll. All other chemicals were analytical grade compounds obtained from commercial sources.

Animals. Young male Wistar strain rats were obtained from the animal breeding colony of the Postgraduate Institute of Medical Education and Research. The animals were acclimatized to laboratory conditions for a few days before commencement of the experiments. All animals were housed individually in plastic cages with stainless steel lids and were fed rat chow (Hindustan Lever, Bombay, India) containing 20% protein, 0.7% Ca\(^{2+}\), 0.5% phosphorus, 0.004% Zn\(^{2+}\), and tap water ad libitum. Zn\(^{2+}\) content in the diet was measured by atomic absorption spectrophotometry as described earlier (42).

The rats were randomly segregated into three groups as follows: hypothyroid (Hypo-T), hyperthyroid (Hyper-T), and euthyroid (Eu-T). Hypo-T and Hyper-T status was induced essentially as described by Kinsella and Sacktor (26). Hypo-T rats were fed thiouracil added to the chow (3 g/kg) and water (0.25 g/l). Hyper-T rats were fed thyroid powder (1–2 g/kg) added to the thiouracil-containing chow. All the rats were kept on the test diets for 3 wk. At the end of the study, the animals were killed under light ether anesthesia, and aortic blood was collected. Serum concentrations of T\(_3\) and T\(_4\) were determined in each group by the method of Lowry et al. (31) after solubilization of the sample in 2% sodium lauryl sulfate as described earlier (39). The protein yields in intestinal and renal BBM isolated from Eu-T, Hypo-T, and Hyper-T rats were similar (4–5 mg/g intestinal mucosa; 7–8 mg/g kidney cortex).

SDS-PAGE. SDS-PAGE of intestinal and renal BBMV proteins was performed by the method of Laemmli (28) using a MINI-PROTEAN II electrophoresis apparatus (Bio-Rad). The separating gel contained 10% (wt/vol) acrylamide and 0.23% bis-acrylamide before polymerization, and resolving gel was of 5% polyacrylamide. Intestinal and renal BBMV samples (6 µg protein) were dissolved in 25 µl of 0.625 M Tris·HCl, pH 6.8, containing 20% (wt/vol) glycerol, 1% SDS (wt/vol), 25 mM β-mercaptoethanol, and 0.05% bromphenol blue and were analyzed by electrophoresis. Broad-range molecular weight markers (Sigma) were also run along with the test samples. Electrophoresis was performed at 10 mA/0.75 mm gel for ~1.5 h until the tracking dye reached the lower end of the gel. After the electrophoresis, the protein bands were visualized by the silver staining procedure (32).

Transport measurements. Uptakes of Zn\(^{2+}\) and D-glucose were measured at 22°C by the Millipore filtration technique using 0.45-µm filters (22, 39). For Zn\(^{2+}\) uptake, 10 µl of BBMV (80–120 µg protein) prepared in 300 mM mannitol and 15 mM HEPES-KOH (pH 6.8) were incubated in 40 µl of uptake buffer containing 300 mM mannitol, 15 mM HEPES-KOH (pH 6.8), 1 mM ZnCl\(_2\), and 1.0 µCi \(^{65}\)Zn\(^{2+}\). The uptake was terminated by the addition of 3 ml of ice-cold stop solution consisting of 150 mM KCl, 15 mM HEPES, and 5 mM EGTA (pH 6.8). The filters were rinsed twice with this solution. Radioactivity retained on the filters was measured by an

### Table 1. Metabolic parameters in hypo-, hyper-, and euthyroid rats

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Body Weight, g</th>
<th>Food Intake, g/day</th>
<th>Serum T(_3), µg/dl</th>
<th>Serum T(_4), µg/dl</th>
<th>Serum Creatinine, mg/dl</th>
<th>Creatinine Clearance, ml/min</th>
<th>GFR/100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo-T</td>
<td>15</td>
<td>166.5 ± 4.7</td>
<td>192 ± 5.7</td>
<td>17.8 ± 2.2</td>
<td>0.5 ± 0.08*</td>
<td>2.57 ± 0.45*</td>
<td>2.2 ± 0.2</td>
<td>0.33 ± 0.08</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>Hyper-T</td>
<td>16</td>
<td>167.2 ± 4.5</td>
<td>120 ± 5.9b†</td>
<td>26.7 ± 2.3†</td>
<td>4.05 ± 0.50†</td>
<td>11.50 ± 2.0†</td>
<td>2.1 ± 0.1</td>
<td>0.73 ± 0.10†</td>
<td>0.61 ± 0.08†</td>
</tr>
<tr>
<td>Eu-T</td>
<td>12</td>
<td>166.0 ± 4.0</td>
<td>195 ± 6.0</td>
<td>17.6 ± 2.1</td>
<td>1.90 ± 0.43</td>
<td>4.88 ± 0.29</td>
<td>1.95 ± 0.1</td>
<td>0.43 ± 0.07</td>
<td>0.22 ± 0.04</td>
</tr>
</tbody>
</table>

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<tr>
<th>Status</th>
<th>n</th>
<th>Food Intake, g/day</th>
<th>Serum T(_3), µg/dl</th>
<th>Serum T(_4), µg/dl</th>
<th>Serum Creatinine, mg/dl</th>
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<td>1.95 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. T\(_3\), triiodothyronine; T\(_4\), thyroxine; GFR, glomerular filtration rate; Hypo-T, hypothyroid; Hyper-T, hyperthyroid; Eu-T, euthyroid. Statistical analysis was conducted using preplanned orthogonal contrast following ANOVA. *F-values having P < 0.05 compared with Eu-T and Hypo-T rats, respectively, were considered significant.

### Table 2. Zn\(^{2+}\) and metallothionein contents in intestine, kidney, and liver of hypo-, hyper-, and euthyroid rats

<table>
<thead>
<tr>
<th>Status</th>
<th>Intestine</th>
<th>Kidney Cortex</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn(^{2+})</td>
<td>MT</td>
<td>Zn(^{2+})</td>
</tr>
<tr>
<td>Hypo-T</td>
<td>24.0 ± 1.5*</td>
<td>88 ± 4.2*</td>
<td>15.62 ± 1.4*</td>
</tr>
<tr>
<td>Hyper-T</td>
<td>43.6 ± 2.1†</td>
<td>150 ± 9.1†</td>
<td>35.01 ± 2.5†</td>
</tr>
<tr>
<td>Eu-T</td>
<td>35.0 ± 3.3</td>
<td>110 ± 6.0</td>
<td>24.7 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats. Zn\(^{2+}\) and metallothionein (MT) contents in intestine, kidney cortex, and liver are expressed as µg/g wet tissue. Statistical analysis was conducted using preplanned orthogonal contrasts following ANOVA. *F-values having P < 0.05 compared with Eu-T and Hypo-T rats, respectively, were considered significant.
Fig. 1. SDS-PAGE of renal (A) and intestinal (B) brush-border membrane (BBM) proteins. Protein (6 µg) was applied in each lane and resolved on 10% polyacrylamide gel. The gel was silver stained. A: lane 1, standard molecular mass markers; lane 2, hyperthyroid rats (Hyper-T); lane 3, euthyroid rats (Eu-T); lane 4, hypothyroid rats (Hypo-T). B: lane 1, Eu-T; lane 2, Hyper-T; lane 3, Hypo-T. Symbols + and − denote increase and decrease, respectively, in intensity of bands in the indicated lane compared with other two lanes. *Band visualized in the indicated lane but not in other lanes.

autogamma scintillation counter (1282 CompuGamma, Universal Gamma Counter). For glucose uptake, 10 µl of the membrane suspension (80–120 µg of protein) were preincubated at 22°C for 1 min, and uptake was initiated by addition of 40 µl of uptake medium containing a final concentration of 150 mM NaCl, 10 mM HEPES-KOH, and 25 µM glucose (0.1 µCi [U-14C]glucose), pH 7.5. The uptake was terminated by addition of 30 vol of ice-cold uptake medium followed by two washings. The filters were then counted for radioactivity using liquid scintillation spectrometry (22).

Fluorescence polarization studies. The steady-state fluorescence anisotropy (r) of DPH in BBM samples was measured using a polarization spectrophotometer (24). In brief, BBM samples were diluted with a phosphate and HEPES-buffered saline (pH 7.4) to a concentration of 0.4, 0.2, and 0.1 mg protein/ml. One microliter of DPH was then added from a 1 mM stock solution in tetrahydrofuran, and the sample was vortexed vigorously. The suspension was incubated for 30 min at 37°C. Fluorescence was measured at an excitation wavelength of 360 nm and a emission wavelength of 430 nm. All measurements were done in a 3-mm square quartz cuvette that minimizes the depolarization of the emitted light due to scattering. The circulating water bath was used to regulate the temperature of membrane suspension within ±0.1°C. The intensities of the parallel (I) and perpendicular (I) components of the emission were measured for both parallel and perpendicular components of excitation wavelength. The fluorescence (P) of fluorescence was obtained from the values of intensity by the following relation: P = I/I/I (34). The anisotropy parameter (r = I/I/I − 1−1) was calculated using the limiting anisotropy of DPH (r0 = 0.362; see Ref. 46). The apparent microviscosity (n) of the lipid region of the membrane preparation was estimated using the modification of the Perrin equation (46), whereby approximate expression for n can be obtained by n = 2P/0.46 − P = 2.4 r/0.362 − r = I/I/I − 10.73 − 0.27 I/I/I (8).

Phase transition in lipids is characterized by an abrupt change with temperature; therefore, Zn2+ uptake was measured at 10, 15, 20, 25, 30, 35, and 40°C in the presence of 1 mM 65Zn2+ as described in Transport measurements. The activation energy was calculated using the Arrhenius equation as described earlier (39).

Statistical analysis. The statistical analysis was done by using one-way ANOVA. Significance was calculated using preplanned orthogonal contrasts comparing two groups. F values having a P < 0.05 were considered significant.

RESULTS
Metabolic effects in relation to thyroid hormone status. As shown in Table 1, serum T3 and T4 levels were reduced significantly in Hypo-T rats compared with Hyper-T and Eu-T rats. The body weights of Hyper-T rats were significantly lower than those of Hypo-T and Eu-T rats. There was no significant difference in body weights between Hypo-T and Eu-T rats. In spite of decreased body weight, the food intake was significantly increased in Eu-T rats compared with Hypo-T and Hyper-T rats. This alteration in food intake in different groups was associated with basal metabolic rate (16). We measured endogenous creatinine clearance as an index of GFR.

Table 3. Zn2+ uptake in intestinal and renal BBMV isolated from hypo-, hyper-, and euthyroid rats

<table>
<thead>
<tr>
<th>Status</th>
<th>Intestine</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min</td>
<td>2 h</td>
</tr>
<tr>
<td>Hypo-T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−) A-23187</td>
<td>17.83 ± 1.02*</td>
<td>66.70 ± 3.45</td>
</tr>
<tr>
<td>(+) A-23187</td>
<td>27.89 ± 1.31*</td>
<td>88.91 ± 3.93</td>
</tr>
<tr>
<td>Hyper-T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−) A-23187</td>
<td>40.29 ± 3.44†</td>
<td>87.50 ± 3.52†</td>
</tr>
<tr>
<td>(+) A-23187</td>
<td>54.37 ± 3.88†</td>
<td>93.23 ± 4.95</td>
</tr>
<tr>
<td>Eu-T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−) A-23187</td>
<td>26.30 ± 2.23</td>
<td>79.3 ± 3.99</td>
</tr>
<tr>
<td>(+) A-23187</td>
<td>38.46 ± 2.98</td>
<td>85.3 ± 3.42</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE of 6 independent preparations. For initial (1 min) and equilibrium (2 h) uptake, the brush-border membrane vesicles (BBMV) reconstituted in 15 mM HEPES and 300 mM mannitol (pH 6.8) were incubated in the uptake medium containing 1 mM 65Zn2+, 15 mM HEPES, and 300 mM mannitol (pH 6.8) in the presence (+) or absence (−) of 10 µM ionophore A-23187. Uptakes were terminated, and radioactivity was retained on the filter was counted as described in MATERIALS AND METHODS. Statistical analysis was conducted using preplanned orthogonal contrasts following ANOVA. ††F-values having P < 0.05 compared with Eu-T and Hypo-T rats, respectively, were considered significant.
GFR and GFR per 100 gram body weight were increased significantly in Hyper-T rats compared with either Hypo-T or Eu-T rats, indicating a substantial increase in filtered load. However, there was no significant change in serum creatinine levels in either group.

Effect of thyroid hormone status on Zn$^{2+}$ and MT content in intestine, liver, and kidney. Zn$^{2+}$ and MT contents in intestine, liver, and kidney cortex of different groups are presented in Table 2. Zn$^{2+}$ content in the intestine, liver, and kidney cortex of Hyper-T rats was significantly higher than the corresponding organs of Hypo-T and Eu-T rats. Zn$^{2+}$ content in these organs of Hypo-T rats was significantly lower than that in Eu-T rats. These observations suggest that accumulation of Zn$^{2+}$ in intestine, liver, and kidney cortex varies in parallel to the thyroid hormone status of the animals. MT levels were found in proportion to the Zn$^{2+}$ content in these organs of different groups, reflecting the induction of MT either in response to Zn$^{2+}$ status of the cell or to thyroid hormone status.

SDS-PAGE of BBM protein. A comparison of BBM protein pattern from the three groups based on SDS-PAGE is shown in Fig. 1. It showed striking similarity with respect to number of bands recovered and their electrophoretic mobilities. However, intensity of some bands varied between the groups, indicating that thyroid hormone status altered the expression of various proteins in intestinal and renal BBM.

Effect of thyroid hormones on Zn$^{2+}$ uptake. Zn$^{2+}$ transport activity in intestinal and renal BBMV was affected by the thyroid hormone status of the rats from which the membranes were derived (Table 3). Hyper-T rats showed a significant increase in the initial (1-min) uptake in both the intestinal and renal BBMV compared with Hypo-T and Eu-T rats. In contrast, Hypo-T rats showed a significant decrease in initial uptake of Zn$^{2+}$. Interestingly, equilibrium (2-h) uptake was also significantly higher in the Hyper-T rats in intestinal and renal BBMV compared with Hypo-T and Eu-T rats. This finding indicated a probable alteration in the intravesicular volume of the BBMV in different groups, which could have been responsible for the altered initial uptake of Zn$^{2+}$ observed in these groups. To find out whether intravesicular volume is the factor for a
significant accumulation of Zn$^{2+}$ at 2 h in intestinal and renal BBMV of Hyper-T rats, initial and equilibrium uptake measurements were carried out in the presence or absence of 10 µM A-23187 in the uptake buffer. In the presence of the ionophore, initial uptake of Zn$^{2+}$ by intestinal and renal BBMV was increased to the same extent in all three groups. However, the initial uptake remained significantly different in these groups. In contrast, equilibrium uptake values in the presence of the ionophore were found not to be significantly different in either of the groups. These findings indicated that the intravesicular volume of BBMV prepared either from the intestinal mucosa or renal cortex of the Eu-T, Hypo-T, and Hyper-T rats was similar.

Effect of thyroid hormone status on kinetic constants of Zn$^{2+}$ transport systems. Next, the kinetic properties of the Zn$^{2+}$ transport in intestinal and renal BBMV were examined. The initial uptake of Zn$^{2+}$ in the intestinal and renal BBMV was measured at different concentrations of Zn$^{2+}$ in the uptake buffer (Figs. 2A and 3A). Hyper-T rats showed a significant increase in the maximal Zn$^{2+}$ transport activity ($V_{\text{max}}$) in the intestinal and renal BBMV compared with Hypo-T and Eu-T rats. Hyper-T rats, on the other hand, showed a $V_{\text{max}}$ significantly lower than even the Eu-T rats (Table 4). However, thyroid hormone status did not alter the apparent Michaelis constant ($K_{\text{m}}$) in the intestinal and renal BBMV in either of the groups.

Table 4. Kinetic constants of Zn$^{2+}$ transport systems in intestinal and renal BBM of hypo-, hyper- and euthyroid rats

<table>
<thead>
<tr>
<th>Status</th>
<th>$V_{\text{max}}$, nmol·min$^{-1}$·mg protein$^{-1}$</th>
<th>$K_{\text{m}}$, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intestinal BBM</td>
<td>Renal BBM</td>
</tr>
<tr>
<td>Hypo-T</td>
<td>45.2±2.8*</td>
<td>25.1±1.2*</td>
</tr>
<tr>
<td>Hyper-T</td>
<td>88.4±4.0†</td>
<td>39.3±2.7†</td>
</tr>
<tr>
<td>Eu-T</td>
<td>63.1±3.8</td>
<td>30.4±1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 independent preparations. $V_{\text{max}}$, maximal velocity; $K_{\text{m}}$, Michaelis constant; BBM, brush-border membrane. Statistical analysis was conducted using preplanned orthogonal contrasts following ANOVA. *†F-values having $P < 0.05$ compared with Eu-T and Hypo-T rats, respectively, were considered significant.

Effect of temperature on the Zn$^{2+}$ transport systems in intestinal and renal BBMV isolated from different groups was carried out to correlate temperature-dependent changes in the activities of the transmembrane proteins and the physical state of the membrane lipids. The temperature dependence of Zn$^{2+}$ transport activity was expressed as an Arrhenius plot ($\log V$ vs. $1/k$). The linear plots with two slopes were observed in intestinal and renal BBMV from the three groups (Fig. 4). There was a significant increase in the transition temperatures of intestinal and renal BBM of Hypo-T rats compared with Hyper-T rats (Table 5). However, no significant change was found in transition temperature of intestinal and renal BBM between Hyper-T and Eu-T rats. The flow of activation energy below and above the transition temperature was not significantly different in Hypo-T, Hyper-T, and Eu-T rats.

Fluorescence polarization studies. The fluorescence polarization and anisotropy of DPH are inversely related to membrane fluidity. These parameters were significantly lower in intestinal and renal BBM from Hyper-T rats than from Eu-T and Hypo-T rats (Fig. 5). The values of apparent microviscosity and anisotropy parameter $[(r_{i}r_{f}) - 1]^{-1}$ obtained at 37°C from intestinal and renal BBM isolated from Hyper-T rats were significantly lower than those from Eu-T and Hypo-T rats (Fig. 6). The lower microviscosity of intestinal and renal BBM from Hyper-T rats could then be interpreted as an increased fluidity of their lipids.
Table 5. Activation energies and thermotropic transition temperature of intestinal and renal BBM of hypo-, hyper- and euthyroid rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intestinal BBM</th>
<th>Renal BBM</th>
<th>Intestinal BBM</th>
<th>Renal BBM</th>
<th>Intestinal BBM</th>
<th>Renal BBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>δE₁, kcal/mol</td>
<td>10.17 ± 0.56</td>
<td>7.22 ± 0.51</td>
<td>13.82 ± 0.89</td>
<td>8.47 ± 0.83</td>
<td>11.93 ± 0.74</td>
<td>7.45 ± 0.66</td>
</tr>
<tr>
<td>δE₂, kcal/mol</td>
<td>5.82 ± 0.13*</td>
<td>2.84 ± 0.12</td>
<td>4.81 ± 0.15</td>
<td>3.03 ± 0.22</td>
<td>4.41 ± 0.31</td>
<td>3.24 ± 0.28</td>
</tr>
<tr>
<td>Tₜ, °C</td>
<td>37.0 ± 1.18*</td>
<td>33.1 ± 0.85*</td>
<td>27.2 ± 0.85†</td>
<td>27.1 ± 0.81†</td>
<td>30.12 ± 0.56</td>
<td>28.89 ± 0.43</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 independent experiments. Activation energies were determined from Arrhenius plots (Fig. 3). δE₁,δE₂, Activation energies below/above transition temperature, respectively; Tₜ, transition temperature. Statistical analysis was conducted using preplanned orthogonal contrasts following ANOVA. *†P-values having P < 0.05 compared with Eu-T and Hypo-T rats, respectively, were considered significant.

Thyroid hormone and Na⁺-dependent uptake of D-glucose. To examine the specificity of the effect of thyroid hormone on Zn²⁺ transport activity, Na⁺-dependent uptake of D-glucose was also determined in membrane vesicles from Hypo-T, Hyper-T, and Eu-T rats (Fig. 7). The initial (30-s) uptake of Na⁺-dependent D-glucose was similar in either of the groups. Additionally, there was no change in passive (Na⁺-independent) transport of D-glucose in these groups (data not shown). Equilibrium (60-min) uptakes of D-glucose in the three groups were similar in intestinal and renal BBMV, substantiating our earlier finding that there was no change in the intravesicular volume of the BBMV prepared from either group.

DISCUSSION

The present study demonstrated that thyroid hormones alter the Zn²⁺ transport activity in both intestinal and renal BBMV. The membranes from Hypo-T rats had reduced Zn²⁺ uptake activity in contrast to Hyper-T rats, which had greater activity compared with preparation from Eu-T rats. The mechanism by which T₃ and T₄ regulate Zn²⁺ uptake is not known. However, incubating the intestinal and renal BBMV from the Eu-T rats with T₃ and T₄ (up to 50 nM) in vitro for 1 h at 25°C did not alter the Zn²⁺ uptake significantly (data not shown). Presumably, the intact cell was necessary to demonstrate the effect of thyroid hormones on Zn²⁺ transporters. Thyroid hormone is known to exert cellular effects through binding to a receptor protein located within the nucleus of target tissues such as intestine, kidney, and other tissues. These receptors are expressed in a highly regulated tissue-specific manner (20, 21, 53). Na⁺-Pₐ cotransport and Na⁺/H⁺ exchanger (NHE) activity in renal BBMV have been correlated with thyroid hormone status of animals (13, 26). Recently, T₃ has been shown to regulate the renal NHE3 and NHE2 isom form mRNAs, both likely to be apical exchangers (2).

Our results demonstrated that the Hyper-T state increased Vₘₐₓ for Zn²⁺ without affecting Kₘ in either intestinal or renal BBMV. The thyroid hormone-mediated increase in Zn²⁺ uptake may be attributable to the following two factors: 1) increase in number/turnover of Zn²⁺ transporter and 2) alteration in the lipid environment leading to conversion of inactive transporters into active ones. Indeed the increased Vₘₐₓ could be associated with increased expression of protein involved in Zn²⁺ transport, as thyroid hormones are known to exert the cellular effects through receptors, that specifically recognize and bind DNA sequence, thereby acting at the transcription level (37). In our study, we have also observed a different pattern of protein expression in renal and intestinal BBMV on SDS-PAGE in all three groups. However, it was not possible to pinpoint whether the expression of protein involved in Zn²⁺ transport is increased or not, since none of the Zn²⁺ transporters in the intestinal and renal BBMV have been characterized to date. Recently, in the human intestinal cell line, Zn²⁺ transport like Ca²⁺ has been shown to occur via both saturable and nonsaturable processes; in addition, lysosome-mediated transcellular movement of Zn²⁺ has also been suggested (14). Vitamin D has been shown to increase Zn²⁺ transport across the cell with a simultaneous increase in MT content. T₃ in vitro has been...
shown to potentiate the genomic effects of vitamin D on Ca\(^{2+}\) and Pi transport in chick intestine (10). Thus it is possible that the thyroid hormone-mediated increase in Zn\(^{2+}\) transport activity in intestinal and renal BBM occurs as a result of interaction between thyroid hormones and vitamin D. Schräd er et al. (44) demonstrated that T\(_3\) modulates the vitamin D-mediated expression of human osteocalcin and mouse osteopontin genes. Therefore, the observed changes in \(V_{\text{max}}\) of Zn\(^{2+}\) transport activity could be a result of interaction of thyroid hormone and vitamin D at genomic levels manifesting itself as increased expression of protein involved in Zn\(^{2+}\) transport in intestinal and renal BBM.

However, a strong correlation has also been observed between the \(V_{\text{max}}\) and membrane fluidity. Indeed thyroid hormones appear to stimulate virtually all aspects of lipid metabolism, including synthesis, mobilization, and degradation (1, 27, 47). In general, thyroid hormone excess is associated with a decrease in stores of most lipids, including triglycerides, phospholipids, and cholesterol. Thyroid hormones have also been shown to change the lipid composition of rat colonic plasma membrane (7). It is well accepted that membrane undergoes many functional changes when subjected to various intrinsic and extrinsic stimuli by modification of its physical state (4). The sensitivity of Zn\(^{2+}\) transport in intestinal and renal BBM to changes in its lipid environment is evident from the observations of Arrhenius plots (Fig. 4). Existence of breaks at different temperatures in Hyper-T and Hypo-T rats suggests a lipid phase separation within the membrane. Increased fluidity of intestinal and renal BBM from Hyper-T rats is also associated with a decrease in transition temperature (Table 5). Fluorescence anisotropy of DPH, inversely related to membrane fluidity, denotes the structural and dynamic properties that determine the relative motions and order of lipid molecules in the membrane (9, 51). The membrane fluidity can modulate transport processes at different levels, i.e., accessibility and translocation steps. In both of the steps, the \(V_{\text{max}}\) of the transport system can be modified even without changing the total number of carriers. A direct effect of membrane fluidity on the accessibility of protein and freedom of protein conformational changes has been documented (4, 6, 33). In isolated BBM and cultured renal cells, a moderate

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**Fig. 6.** Anisotropy parameter \(\frac{[r_0]}{r_2}\) and microviscosity \((\eta, \text{poise})\) in intestinal and renal BBM isolated from Eu-T, Hypo-T, and Hyper-T rats. All values are means ± SD of 6 independent experiments. Statistical analysis was conducted using preplanned orthogonal contrast after ANOVA. * and § \(F\) values having \(P < 0.05\) compared with Eu-T and Hypo-T rats, respectively, were considered significant.

**Fig. 7.** Effect of thyroid hormones on Na\(^{+}\)-dependent D-glucose transport in intestinal (A) and renal (B) BBMV from Hyper-T, Hypo-T, and Eu-T rats. D-Glucose (25 µM) uptake in BBMV was measured as the difference between glucose uptake in the presence of an initial Na\(^{+}\) gradient (extracellular Na\(^{+}\) concentration = 150 mM, intracellular Na\(^{+}\) concentration = 0 mM) and an initial K\(^{+}\) gradient (extracellular K\(^{+}\) concentration = 150 mM, intracellular K\(^{+}\) concentration = 0 mM). Values are expressed as mean ± SE of 6 experiments.
fluidization stimulates the Na\(^{+}\)-phosphate transport (15, 55). This stimulation resulted from an increase in the \(V_{\text{max}}\) of the transport system, leaving its affinity unchanged. Taken together, these findings are consistent with the present observation that increased Zn\(^{2+}\) uptake is associated with the increase in \(V_{\text{max}}\) due to increased fluidity in Hyper-T rats.

Zn\(^{2+}\) and MT are inextricably linked, and their levels in intestine, kidney, and liver of Hyper-T rats were significantly higher compared with either Hypo-T or Eu-T rats. The MT occurs in vivo with its component of bound metal. The 5'-untranslated region of MT genes in humans and mice contains elements responsive to metals (Cd\(^{2+}\), Zn\(^{2+}\), Hg\(^{2+}\), etc.) and hormones (17). Zn\(^{2+}\), next to Cd\(^{2+}\), is the most successful MT-inducing agent. Generally, a close relationship exists between Zn\(^{2+}\) status and levels of MT, as observed in the present study; however, direct action of thyroid hormones in induction of MT is not yet known.

Further studies are required to elucidate the effect of thyroid hormones on the expression of proteins involved in transport and storage of Zn\(^{2+}\) in intestine and kidney. However, the above evidence that thyroid hormones modulate Zn\(^{2+}\) transport activity in intestinal and renal BBM is of importance, as Zn\(^{2+}\) is a very potent trace element, and its transporter response to the hormones provides attractive models to examine the cellular and molecular mechanisms for endocrine control of transport processes.

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REFERENCES


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