Glucose transporter 2 concentrations in hyper- and hypothyroid rat livers

Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Aichi 470–1192, Japan
(Requests for offprints should be addressed to A Nagasaka)

Abstract
The deterioration of glucose metabolism frequently observed in hyperthyroidism may be due in part to increased gluconeogenesis in the liver and glucose efflux through hepatocyte plasma membranes. Glucose transporter 2 (GLUT 2), a facilitative glucose transporter localized to the liver and pancreas, may play a role in this distorted glucose metabolism.

We examined changes in the levels of GLUT 2 in livers from rats with l-thyroxine-induced hyperthyroidism or methimazole-induced hypothyroidism by using Western blotting to detect GLUT 2.

An oral glucose tolerance test revealed an oxyhyperglycemic curve (impaired glucose tolerance) in hyperthyroid rats (n=7) and a flattened curve in hypothyroid rats (n=7). GLUT 2 levels in hepatocyte plasma membranes were significantly increased in hyperthyroid rats and were not decreased in hypothyroid rats compared with euthyroid rats. The same results were obtained with a densitometric assay. These findings suggest that changes in the liver GLUT 2 concentration may contribute to abnormal glucose metabolism in thyroid disorders.


Introduction
The homeostasis of glucose metabolism is maintained by many factors, including intestinal glucose absorption, liver gluconeogenesis and glucogenolysis, muscle glucose uptake, insulin secretion from pancreatic β cells, and anti-insulinogenic hormones, e.g. cortisol and thyroid hormones. It is well known that an impaired glucose tolerance is induced by the excessive hormone secretion seen in various endocrinopathies.

In hyperthyroidism, impaired glucose tolerance (IGT) is frequently observed after oral glucose loading. IGT in hyperthyroidism is thought to be caused by abnormal glucose metabolism in muscle, fat and liver (Saunder et al. 1980), or by the sluggish secretion of insulin (Lenzen & Kücking 1982) and accelerated degradation of plasma insulin (Doar et al. 1969), and/or by increased glucose absorption by a hyperkinetic gastrointestinal tract (Scarf 1936). Increased gluconeogenesis and excessive efflux of glucose from hepatocytes are thought to contribute to impaired glucose tolerance (Menahan & Wieland 1969). In hepatic gluconeogenesis and glucolysis, glucose is rapidly transported across the hepatocyte plasma membrane. In this glucose transport system, the efflux of glucose across the hepatocyte plasma membrane is the final step in hepatic glucose production, and is largely mediated by glucose transporter 2 (GLUT 2), a facilitative glucose transporter (Elbrink & Bihler 1975). Thus, GLUT 2, which is localized to the liver and pancreas (Fukumoto et al. 1988), may be related to the abnormal glucose metabolism seen in hyperthyroidism. In this study we examined changes in GLUT 2 levels in livers from hyper- and hypothyroid rats by Western blot analysis.

Materials and Methods

Preparation and specificity of antibody
The COOH-terminal 16-amino acid peptide (Arg-Lys-Ala-Thr-Val-Gln-Met-Glu-Phe-Leu-Gly-Ser-Ser-Glu-Thr-Val) of rat GLUT 2 (Thorens et al. 1988) was synthesized with a peptide synthesizer (Model 430A, Applied Biosystems, Foster, NJ, USA) and purified to 96% purity by high performance liquid column chromatography (HPLC). The rabbits were immunized with 5 mg GLUT 2 peptide conjugated to 5 mg bovine serum albumin (BSA) with the use of glutaraldehyde, and exsanguinated after 10 weeks. The crude immunoglobulin G (IgG) fraction of the antisera was prepared by ammonium sulfate precipitation and diluted to 1:200. The anti-GLUT 2 peptide antibody reacted with a 45 kDa protein in hepatocyte membranes, but not with proteins from skeletal


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muscle. On preincubating the antibody with the excess GLUT 2 peptide for 5 min, the 45 kDa band in liver cell membranes disappeared (Fig. 1b, L), suggesting that the anti-GLUT 2 peptide antibody specifically recognized GLUT 2 as an antigen.

Induction of hypo- or hyperthyroidism

Fourteen male Wistar rats (7 weeks old, weighing approximately 280 g) received 1 mg 1-methyl-2-mercaptoimidazole (MMI) diluted with distilled water to drink every day in order to produce a hypothyroid state. Four weeks after the start of MMI treatment, half of the rats in the hypothyroid state received daily, by eye-dropper, 200 µg T4 diluted with saline to induce a hyperthyroid state, while the other rats received saline without T4. Control rats (n=7) drank saline daily without T4 or MMI. The total treatment period for each group was 8 weeks. The rats’ body weights, blood glucose concentrations, serum fructosamine concentrations, and serum tri-iodothyronine (T3) and T4 concentrations were measured at the start and 4 and 8 weeks after the treatment, and compared with those in control rats. After 8 weeks, an oral glucose tolerance test (GTT) was performed for rats fasted for 16 h before the test. Glucose (2 g/kg) diluted with water to a 50% glucose concentration was orally administered to each rat, and then blood samples were taken from the tail vein of each rat at 0, 30, 60, 90 and 120 min to measure blood glucose concentrations.

Preparation of liver plasma membranes

As previously reported (Oda et al. 1995), rat liver plasma membranes (PM) were isolated from 20% homogenized livers in STE buffer, pH 7-5 (0·25 M sucrose, 10 mM Tris–HCl, pH 7·5 and 5 mM EDTA) by discontinuous sucrose density gradient ((23-5% sucrose-4% Ficoll):(20% sucrose-1 mM EDTA-10 mM HEPES-Tris buffer, pH 7·4)) centrifugation at 100 000 g for 90 min at 4 °C. The PM fractions were confirmed by assaying 5’-nucleotidase as a plasma membrane marker enzyme.

Western blotting

The PMs solubilized in STE buffer (100 µg protein/lane) were electrophoresed on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and were electroblotted onto nylon membranes (Magnagraph) (Microseparation Inc., Westborough, MA, USA). The filters were blocked with nonfat milk, incubated with the rabbit anti-GLUT 2 antiserum diluted 1:200, washed with Tris–HCl buffer, pH 7·5, and then incubated with peroxidase-conjugated anti-rabbit IgG second antibody (Dako Co., Glostrup, CA, USA). The peroxidase activity was developed with diaminobenzidine/H2O2.

The GLUT 2 band density was measured by densitometric analysis using NIH Image software (US National Institutes of Health, Bethesda, MD, USA).

The protein concentration was determined by a protein assay (Bio-Rad, Hercules, CA, USA) (Bradford 1976).

Differences between the groups were examined with Wilcoxon Rank Sum test or Kruskal–Wallis test. The significance level was P<0·05.

Results

Thyroid and glucose metabolism

The body weights of hyperthyroid rats were significantly decreased at 2 months compared with those of control rats. The thyroid status of these rats was determined by measuring the total serum T4 and T3 concentrations; these were significantly higher in the hyperthyroid rats (P<0·05) and significantly lower in the hypothyroid rats (P<0·05) than in the euthyroid rats. Fructosamine concentrations were not affected in either the hyper- or hypothyroid rats compared with the control rats (Table 1). However, an oral GTT administered to all rats at 8 weeks showed an impaired glucose tolerance curve in the hyper-
thyroid rats, i.e. the glucose level was significantly elevated at 30 min after oral glucose intake, a phenomenon termed oxyhyperglycemia. In the hypothyroid rats, the glucose tolerance curve was slightly flattened compared with control rats (Fig. 2). The areas under the glucose tolerance curves in the hyper- and hypothyroid states are shown in Table 2. The integrated area in the hyperthyroid state was significantly greater than in the euthyroid and hypothyroid states, but did not differ between the hypothyroid and euthyroid states.

**GLUT 2 levels in rat liver plasma membranes**

Western blot analysis of GLUT 2 revealed an increase in GLUT 2 levels in the hyperthyroid rat livers, but no change in GLUT 2 levels in the hypothyroid rat livers. The representative results of six separate experiments, i.e. 2 each of euthyroid, hypothyroid and hyperthyroid rats, are shown in Fig. 3. The other 5 samples from the hyperthyroid rats showed similar results in GLUT 2 levels compared with those from the other euthyroid or hypothyroid rats. The GLUT 2 band densities on all Western blots, measured by densitometric analysis using NIH Image software, were 3 times higher for the hyperthyroid rat livers than for the euthyroid rat livers (Table 3).

**Discussion**

In the present study, the fructosamine concentrations in the hyperthyroid rats were not significantly changed, although the GTT showed oxyhyperglycemia in these rats. Therefore, the changes in the GLUT 2 expressions in the hyperthyroid rat livers may not have been induced by high glucose concentrations, but rather by the action of thyroid hormone, as shown by Weinstein et al. (1994). It has previously been reported that GLUT 2 expression in liver cells is increased when the cells are cultured in the presence of high glucose concentrations (Oka et al. 1990). In our study, GLUT 2 levels were increased in the hyperthyroid rat livers compared with the control rat livers.

Increased GLUT 2 levels in the liver lead to an increase in hepatic glucose transport capacity. As part of the bidirectional glucose transport system in hepatocytes, GLUT 2 has a high $K_m$ for glucose (Williams et al. 1968, Craik & Elliott 1979). Thus, GLUT 2 may play an important role in mediating rapid glucose transport across the hepatocyte plasma membrane. GLUT 2 may act at the final step of glucose output in gluconeogenesis and at the first step of glucose uptake for glycolysis.

### Table 1

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<th>Hyperthyroid</th>
<th>Hypothyroid</th>
<th>Euthyroid</th>
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<tr>
<td>$T_3$ (ng/ml)</td>
<td>2.70 ± 2.12*</td>
<td>0.43 ± 0.11*</td>
<td>0.56 ± 0.13</td>
</tr>
<tr>
<td>$T_4$ (µg/dl)</td>
<td>64.86 ± 11.7*</td>
<td>27.71 ± 2.98*</td>
<td>42.14 ± 4.06</td>
</tr>
<tr>
<td>Fructosamine (µmol/l)</td>
<td>153 ± 11</td>
<td>136 ± 9</td>
<td>146 ± 8</td>
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Rats were treated with MMI (1 mg/day) for 8 weeks (hypothyroid group) or with MMI for 4 weeks and then with MMI and L-thyroxine (200 µg/day) for 4 more weeks (hyperthyroid group). *P<0.05, statistically significant compared with euthyroid rats.

### Table 2

<table>
<thead>
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<th>Hyperthyroid rats</th>
<th>Hypothyroid rats</th>
<th>Euthyroid rats</th>
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<tr>
<td>Integrated area (cm$^2$)</td>
<td>74.7 ± 11.2*</td>
<td>44.5 ± 9.7</td>
<td>57.6 ± 10.4</td>
</tr>
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*P<0.05, statistically significant compared with euthyroid rats.
Therefore, increased GLUT 2 levels may contribute to the oxyhyperglycemia observed in hyperthyroid rats.

Glucose transporter 4 expression is up-regulated in the skeletal muscle of hyperthyroid rats (Weinstein et al. 1991). Similarly, hepatic GLUT 2 may be regulated in the same manner. Thus, glucose transport capacity may be increased in hepatocytes in the hyperthyroid state, causing abnormal glucose metabolism. Therefore, the effects of thyroid hormone on glucose metabolism may be due to changes in glucose transporter levels.

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