Glucose transporter 2 concentrations in hyper- and hypothyroid rat livers

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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.

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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
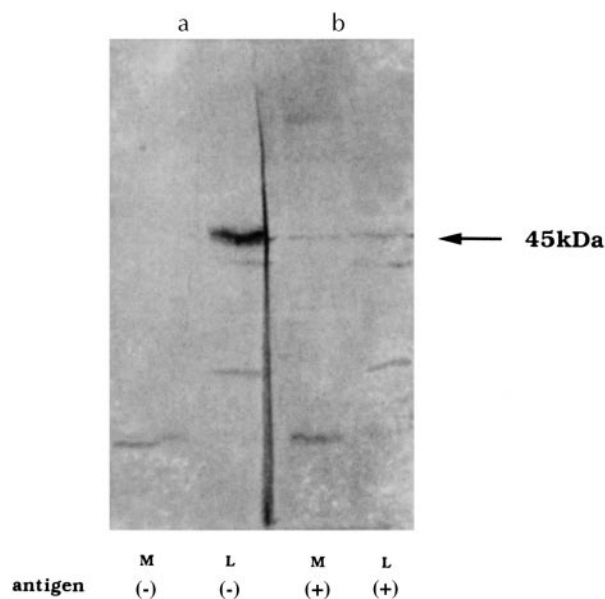
Figure 1 Specificity of GLUT 2 antibody. Anti-GLUT 2 antibody was prepared as described in Materials and Methods section. Immunological reaction of GLUT 2 with the antibody was detected in rat liver (a, L, 45 kDa) but not in rat skeletal muscle (a, M); that is, anti-GLUT 2 antibody specifically reacted to proteins in rat liver. Detection of the GLUT 2 band was blocked when the antibody was preincubated with the COOH-terminal 16 peptide (antigen) for 5 min at room temperature (b, L). L, liver; M, muscle. The antibody preincubated with (+) or without (−) C-terminal GLUT 2 peptide was used.

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 L-thyroxine (T_4) diluted with saline to induce a hyperthyroid state, while the other rats received saline without T_4. Control rats ($n=7$) drank saline daily without T_4 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 (T_3) and T_4 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 dodecylsulfate (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/H_2O_2.

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 T_4 and T_3 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-

Table 1 T_3, T_4 and fructosamine concentrations in hyper-, hypo-, and euthyroid rats at the end of the 8-week treatment period. Each value is the mean ± S.D. for $n=7$ rats examined

	Hyperthyroid	Hypothyroid	Euthyroid
T_3 (ng/ml)	2·70 ± 2·12*	0·43 ± 0·11*	0·56 ± 0·13
T_4 (µg/dl)	64·86 ± 11·7*	27·71 ± 2·98*	42·14 ± 4·06
Fructosamine (µmol/l)	153 ± 11	136 ± 9	146 ± 8

Rats were trated 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 Integrated areas (cm_2) calculated from the glucose tolerance curves in Fig. 2. Each value is the mean ± S.D. for $n=7$ rats examined

	Hyperthyroid rats	Hypothyroid rats	Euthyroid rats
Integrated area (cm^2)	74·7 ± 11·2*	44·5 ± 9·7	57·6 ± 10·4

$P<0.05$, statistically significant compared with euthyroid rats.

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 i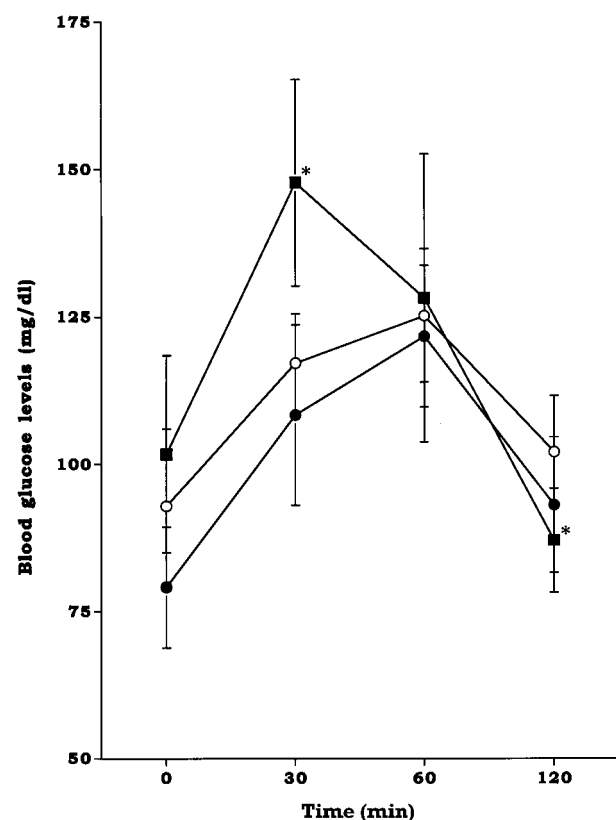n Table 2. The integrated area in the hyperthyroid state was significantly greater than in the euthyroid and hypothyroid states, but did not differ between the hypothyroid and euthyroid states.

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.
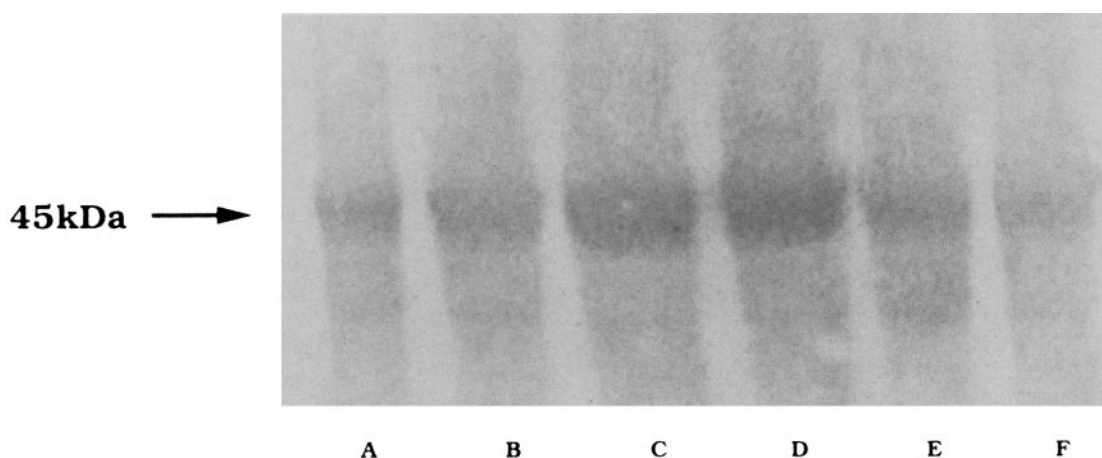

Figure 2 Blood glucose concentrations (mg/dl) during oral glucose (2 g/kg body weight) tolerance testing of hyperthyroid (■), hypothyroid (●) and euthyroid (control) (○) rats. Values are means ± S.D. *$P<0.05$ compared with euthyroid (control) rats.

Figure 3 Western blot analysis of GLUT 2 in liver plasma membranes of hyperthyroid, hypothyroid and euthyroid rats. Liver plasma membranes (100 μg protein/lane) were analyzed for GLUT 2 expression. Arrow (45 kDa) shows GLUT 2 stained by GLUT 2 antibody in the samples from two different euthyroid (A and B), hyperthyroid (C and D) and hypothyroid (E and F) rats.

Table 3 Effect of thyroid status on GLUT 2 protein content (expressed as pixels) in the liver plasma membranes of euthyroid, hyperthyroid and hypothyroid rats. Each value is the mean ± S.D. of seven rats

	Euthyroid	Hyperthyroid	Hypothyroid
GLUT 2	86 029 ± 12 420	266 341 ± 52 130*	145 408 ± 49 628

The GLUT 2 protein concentration was determined as described in the Materials and Methods section. The value in each blotting was adjusted by the value of the saline-treated (euthyroid) rats.
*$P<0.05$, statistically significant compared with that in euthyroid rats.

Therefore, increased GLUT 2 levels may contribute to the oxyhyperglycemia observed in hyperthyroid rats.

Glucose transpoter 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.

Acknowledgements

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