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Role of liver-type glucose transporter (GLUT2) in transport across the basolateral membrane in rat jejunum

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To obtain information on the regulation of glucose transport across the basolateral membrane (BLM) of intestinal epithelial cells, we measured the number of [^{3}H]cytochalasin B binding sites and the level of liver-type glucose transporter (GLUT2) protein in the BLM in the jejunum of rats (i) with diabetes (ii) given a high-carbohydrate diet or (iii) with experimental hyperglycemia (12 h infusion of a high-glucose solution). A glucose uptake and the number of D-glucose inhibitable [^{3}H]cytochalasin B binding sites in BLM vesicles were significantly increased in all three conditions. Western blot analysis showed that the amount of GLUT2 protein in BLM vesicles was increased in rats with diabetes and those given a high-carbohydrate diet, but not in those with experimental hyperglycemia. These results suggest that there is a mechanism for rapid regulation of glucose transport in the BLM that does not depend on change in the amount of GLUT2.

D-Glucose transport; Basolateral membrane; GLUT2; Diabetes; High-glucose infusion; Rat intestine

1. INTRODUCTION

Glucose is transported across the small intestine by a two step process; the first step is entry of glucose into the epithelium across the brush border membrane (BBM), and the second is its efflux from cells across the basolateral membrane (BLM) into the blood [1]. The molecular mechanism of glucose transport across the BBM via the Na^+-dependent glucose co-transporter (SGLT1) has been well characterized by Wright et al. [2], but little is known about glucose transport across the BLM.

Recently, cDNAs for 5 facilitative glucose transporters (GLUT1–5) have been isolated [3]. GLUT2 has been shown to be present in the liver, small intestine, kidney and the islets of Langerhans [4,5]. Thorens et al. suggested that, as GLUT2 has a relative high K_m for glucose, it may be required for glucose sensing by β-cells in islets. This glucose transporter is also present in the BLM of intestinal epithelial cells, and is responsible for the release of glucose across the BLM [4–6]. Gould et al. found that the K_m value for glucose of GLUT2 expressed in *Xenopus* oocytes is consistent with that reported by others for BLM preparations from rat small intestine [7].

Previously we showed that the SGLT1, GLUT2 and GLUT5 genes are expressed in rat jejunum, and that in the jejunum of acute diabetic rats the level of GLUT2 mRNA changed in parallel with the D-glucose transport activity, whereas the level of SGLT1, which is located in the BBM, did not change [8]. These results suggest that the induction of glucose transport occurs in the BLM of intestinal epithelial cells, not in the BBM.

Recently facilitative glucose transport across the BLM was found to be induced by experimental hyperglycemia (infusion of high-glucose solution) or a high-glucose diet [10–13]. However, nothing is yet known about the regulation of the GLUT2 protein in the BLM of intestinal epithelial cells. Therefore, in the present study, we investigated the levels of GLUT2 protein in rats with diabetes, those given high-carbohydrate diet and those with experimental hyperglycemia (12 h infusion of high-glucose solution). Our results clearly indicate differential mechanisms for the regulation of basolateral glucose transport in rat small intestine.

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2. MATERIALS AND METHODS

2.1. *Experimental diabetes*

Male Sprague–Dawley rats (Japan SLC, Shizuoka, Japan), weighing 160–180 g, were used. The animals were housed in stainless-steel cages and given a carbohydrate-free diet (24% protein, 55% fat, 9% cellulose, 4% mineral mixture and 1% vitamins), for 2 weeks before experiments, because the level of dietary sugar significantly affects the level of liver-type glucose transporter (GLUT2) mRNA in rat jejunum [9]. Most diet components were purchased from Wako Chemical Industries (Osaka, Japan). The animals had free access to water and were maintained under a 12-h light:dark cycle at 24°C. Animals were made diabetic by intraperitoneal injection of 65 mg/kg streptozocin (STZ) freshly dissolved in 100 mM citrate buffer (pH 4.5). Control rats

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received a similar injection of citrate buffer only. Animals were given a carbohydrate-free diet and were killed 10 days after STZ injection, as described previously [8].

2.2. *Intravenous infusion experiments*

Intravenous infusion experiments were carried out as described by Maenz and Cheeseman [10,12]. Rats (160–180 g) maintained on a carbohydrate-free diet for 2 weeks were anesthetized with pentobarbital and a tube was inserted into the jugular vein and pushed into the heart. The other end of the tube was brought out through an incision in the back. On the day of use, animals were placed in a small cage and connected to an infusion tube, and a solution of 50% glucose was infused at 6.0 ml/h. After infusion for 12 h, the animals were killed and the jejunum was used for preparation of the BLM.

2.3. *High-carbohydrate diet*

Animals (150–170 g) were maintained for 2 weeks on a carbohydrate-free diet and then given a 55% glucose diet (55% glucose in place of 55% fat) for 5 days before experiments [14].

2.4. *Preparation of BLM from intestinal epithelial cells*

Rats were anesthetized by intraperitoneal injection of pentobarbital, the abdomen was opened and the portal vein was cut. Then warm (37°C) 150 mM phosphate-buffered 0.9% saline, containing 30 mM EDTA, pH 7.4, was injected into the left ventricle, and the small intestine was removed and inverted over a glass rod. Cells were released by shaking, suspended in cold sucrose buffer, and collected by low-speed centrifugation [15]. BLM vesicles were prepared by differential centrifugation and final purification on a Percoll gradient, as described by Scalera et al. [16]. The final BLM pellet showed a 16-fold increase in specific activity of Na^+,K^+-ATPase over that of the crude preparation. There was no significant difference in the Na^+,K^+-ATPase activities in BLM preparations from rats with hyperglycemia and diabetes and those fed a high-carbohydrate diet.

2.5. *Glucose uptake and cytochalasin B binding assay in the BLM*

Glucose transport and binding of cytochalasin B to the BLM from rat jejunum were measured by the methods of Maenz and Cheeseman [11]. Membranes were incubated for 15 min with the ligand in the presence of 2 μM cytochalasin E to reduce non-specific binding. The membranes were pelleted by centrifugation for 30 min and the supernatant was removed before dissolving the pellet in sodium hydroxide. For assay of the glucose-inhibitable cytochalasin B binding, incubations were performed with either 1.25 M D-glucose or 1.25 M sorbitol in the medium. The amount of cytochalasin B bound to BLM vesicles was calculated after correction for unbound [^{3}H]cytochalasin associated with the pellet using [^{14}C]urea as a non-binding aqueous space marker. Binding constants were calculated using a computer program.

In experiments on glucose uptake, 10 μl of vesicles (10 mg protein/ml) was incubated with 20 μl of uptake medium for 3 s and the reaction was stopped with 1,455 μl of ice-cold buffer containing 0.2 mM phloretin. The mixture was promptly filtered through a 0.45 μm pore size filter, which was then washed twice with 5 ml of stop solution [11,12].

2.7. *Western blotting*

GLUT2 antibody was kindly provided by B. Thorens [17]. This antibody was further purified as the IgG fraction by DE-52 cellulose column chromatography. Membranes were resuspended in Laemmli sample buffer containing 5% SDS and separated on a 10% SDS-polyacrylamide gel [18]. Electrotransfer to nitrocellulose filters was performed for 6 h. The filters were washed at room temperature for 10 min in TBST (20 mM Tris, pH 7.4, 150 mM NaCl, and 0.02% Tween 20). The filters were blocked with 0.5% fat-free dry milk, and incubated with anti-peptide antibodies (IgG fraction) against rat GLUT2. They were then washed with TBST at room temperature, and incubated with 10^5 cpm of ^{125}I-labeled protein A/ml TBST. Finally they were washed for 60 min in TBST and exposed to Kodak XAR films at −70°C. Quantification was performed by scanning densitometry.

2.8. *Materials*

D-[^{3}H]glucose, [^{3}H]cytochalasin B, [^{14}C]urea and [^{125}I]protein A were purchased from Amersham. Cytochalasin B, cytochalasin E, D-glucose and D-sorbitol were from Sigma Chemical Co.

3. RESULTS

Membranes isolated from rat jejunum were analyzed for a marker enzyme of the BLM. Their specific activity of Na^+,K^+-ATPase was about 16-fold that of the crude extract (crude homogenate, 9.4 ± 1.8 μmol PO_4/h/mg protein; BLM, 150.9 ± 19.6 μmol PO_4/h/mg protein). GLUT2 antiserum reacted with a band of 61 kDa pro-

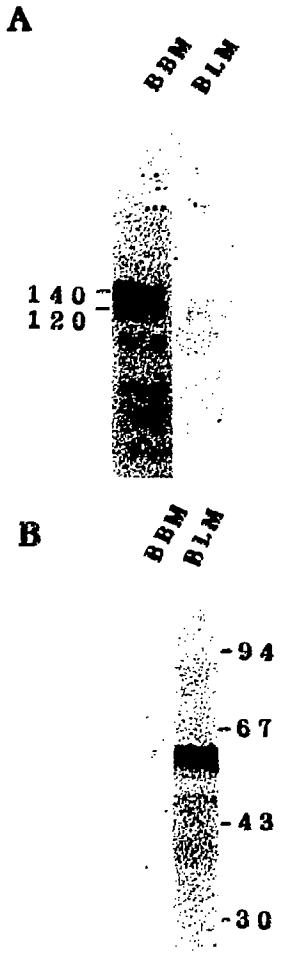

Fig. 1. Western blot analyses of GLUT2 and sucrase in the brush-border (BBM) and basolateral membrane (BLM) of rat jejunum. (A) Sucrase, (B) GLUT2. Intestinal BBM were prepared by the method of Maenz and Cheeseman [10]. The membrane from rat small intestine was purified in the presence of the protease inhibitors, aprotinin, phenylmethylsulfonyl fluoride, *N-p*-tosyllysine chloromethyl ketone, *N*-tosyl-L-phenylalanine chloromethyl ketone and Futhan [5,26]. After separation by SDS-PAGE, the proteins were transferred electrophoretically to a nitrocellulose filter by the method of Towbin et al. [27]. The filter was incubated with anti-GLUT2 and anti-sucrase antibody for 2 h, and then incubated with peroxidase-coupled goat anti-rabbit IgG [5,28]. The bound antibody was located by staining with 4-chloro naphthol.

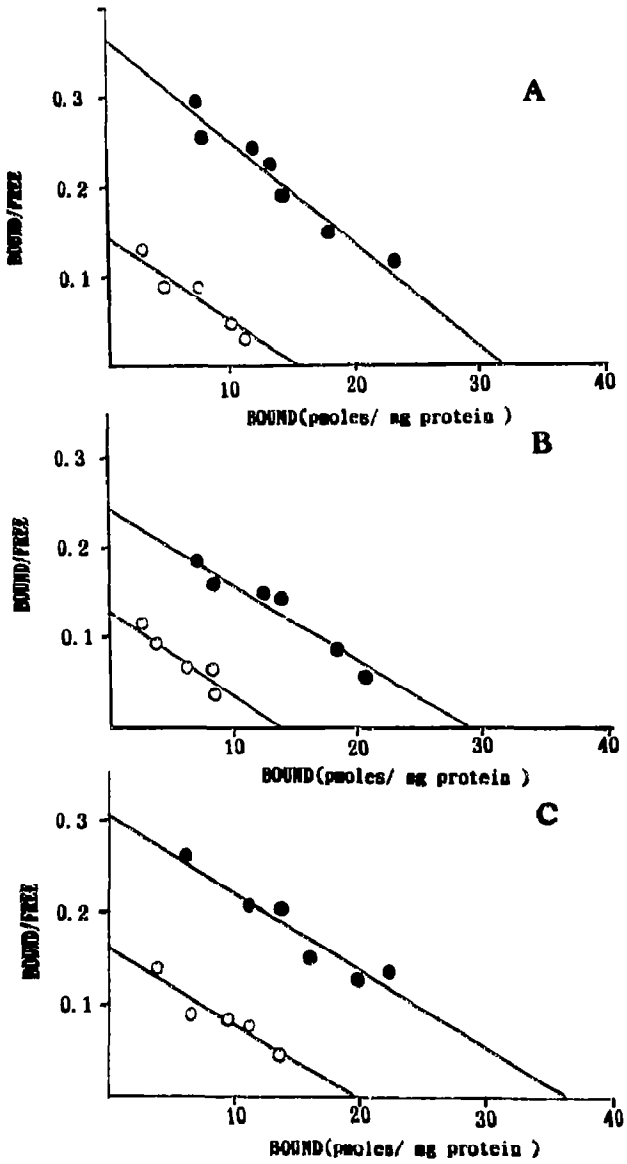

Fig. 2. Scatchard plots of D-glucose-inhibitable [^{3}H]cytochalasin B binding to purified jejunal BLM prepared from rats with hyperglycemia, and diabetes and those fed a high-carbohydrate. D-Glucose-inhibitable [^{3}H]cytochalasin B binding was determined as described in section 2. The data are taken from two typical experiments in which membranes from 8 rats were pooled, and points represent means for triplicate determinations. (A) Diabetes, (B) hyperglycemia, (C) high-carbohydrate. (○) Control group, (●) test group.

tein in the BLM from rat intestinal epithelial cells, but not with any protein in the brush-border or microsomal membranes (Fig. 1). This observation that the molecular weight of intestinal GLUT2 is 61 kDa is consistent with a report of Thorens et al. [17].

Western blotting for sucrase as a marker enzyme of brush-border membranes (BBM) revealed that the preparations were not contaminated with BBM.

Fig. 2 shows the data for [^{3}H]cytochalasin B binding to BLM prepared from rats with diabetes (A) and experimental hyperglycemia (B), and those given a high-carbohydrate diet (C). The number of binding sites were clearly increased in all three groups, and these increases paralleled changes in the V_{max} for transport [10–13]. The V_{max} values for D-glucose-inhibitable cytochalasin B binding in the groups with diabetes and hyperglycemia and those given a high-carbohydrate diet were 30.2 ± 5.1 pmol/mg protein (n = 6), 29.5 ± 4.6 pmol/mg protein (n = 6) and 36.5 ± 6.6 pmol/mg protein (n = 7), the control values being 15.7 ± 3.2 pmol/mg protein, 14.3 ± 4.8 pmol/mg protein and 19.1 ± 3.2 pmol/mg protein, respectively. The binding affinities of these groups were the same: diabetes, 124.7 ± 12.9 μM (control, 119.8 ± 21.8 μM); hyperglycemia, 119.5 ± 21.8 μM (control, 110.6 ± 11.7); high-carbohydrate diet, 121.3 ± 20.8 μM (control, 120.6 ± 17.5 μM). The uptakes of 50 mM glucose in these animals are shown in Fig. 3. D-Glucose transport across the BLM vesicle was significantly increased in all test groups relative to that of the control. This was largely a consequence of an increased V_{max}, not a reduced K_t [10–13]. An increase in [^{3}H]cytochalasin B binding was consistent with the induction of transport observed in BLM preparations.

Previously we found that the level of GLUT2 mRNA changed in parallel with D-galactose transport activity in the jejunum of diabetic rats [8]. As shown in Fig. 4A, the amount of GLUT2 protein increased about 3.0-fold in the BLM vesicles obtained from diabetic rats 10 days after STZ injection. The blood glucose level increased to a maximum 3 days after STZ injection [8]. As shown in Fig. 4C, a high-glucose diet increased in the intestinal level of GLUT2 protein about 2.6-fold after 5 days. The observation that GLUT2 protein increased in rats fed a high-carbohydrate diet is consistent with the results

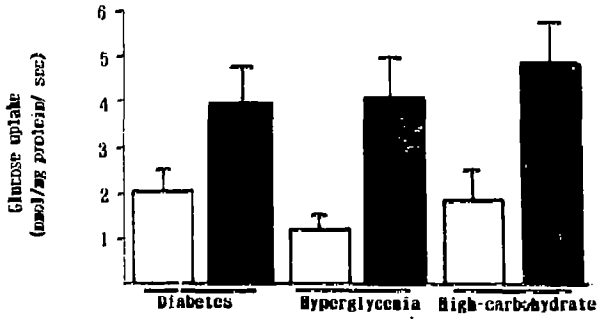

Fig. 3. Glucose uptake across BLM vesicles prepared from rats with hyperglycemia and diabetes and those fed a high-carbohydrate diet. The uptake of glucose into vesicles was initiated by mixing 10 μl of vesicles with 20 μl of uptake media containing D-glucose + D-[^{3}H]glucose [11,12]. Uptake was initiated by vortexing the tube and, at the conclusion of the uptake period, 1,455 μl of ice-cold stop solution was rapidly injected into the mixture. Glucose held by the vesicles was determined as described in section 2. A correction for passive permeation was made by subtracting the uptake of the same concentrations of L-glucose. The bars indicate the S.D. from the mean (n = 6). (A) Diabetes, (B) hyperglycemia, (C) high-carbohydrate. (□) Control group, (■) test group.

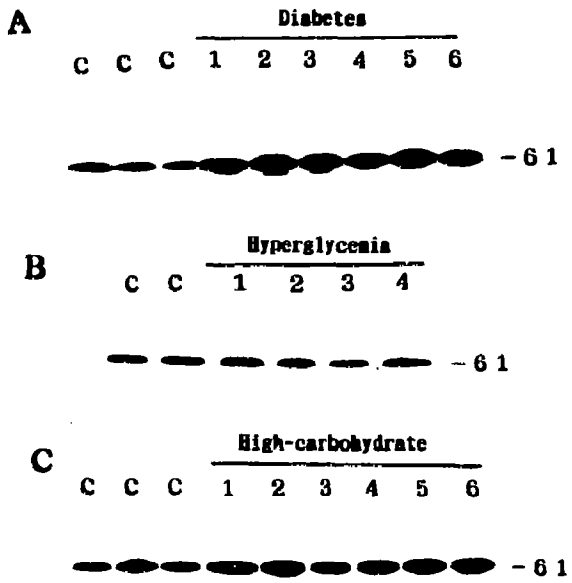

Fig. 4. Western blot of GLUT2 protein in BLM vesicles from rats with hyperglycemia, and diabetes and those fed high-carbohydrate diet. Purified membranes (50 μg of membrane protein/lane) were subjected to SDS-10% PAGE and transferred to nylon filters. The filters were then incubated with anti-peptide antibody of rat GLUT2 and ^{125}I-labeled protein A and subjected to autoradiography. (A) Diabetes, (B) hyperglycemia, (C) high-carbohydrate diet. Lane C, control group; lanes 1–6, test group.

obtained in the cytocalasin B binding experiments [13]. Glucose infusion resulted in significant hyperglycemia (12-h infusion of glucose, plasma glucose = 296 ± 30 mg/ml; saline infusion, 160 ± 19 mg/ml). The effect of a 12-h intravenous infusion of glucose on the level of intestinal GLUT2 protein is shown in Fig. 4B. The GLUT2 protein level was not increased by the 12-h infusion of glucose compared with that of saline infusion, although [^{3}H]cytochalasin B binding increased significantly in these rats (Fig. 2B). We examined whether GLUT2 was present in other membrane fractions in hyperglycemic rats jejunum but detected no immunoreactive band of the protein in brush-border or microsomal membrane preparations. Therefore, we concluded that the GLUT2 protein level did not increase over the control level in rats with hyperglycemia.

4. DISCUSSION

Numerous studies have shown that the transport capacity of the small intestine is altered by a variety of conditions such as change in dietary conditions, lactation, resection, and disease [10–13,19–22]. Many of these adaptational responses take several days and are often explained by alterations in intestinal morphology [1]. However, there are reports that the response of glucose transport to hyperglycemia is a much more rapid type [13,21]. In the present study, we also observed a rapid response of intestinal glucose transport in rats with experimental hyperglycemia (12 h-infusion of high-glucose solution). In these rats, unlike in those with diabetes and those on a high carbohydrate diet, increase in the number of [^{3}H]cytochalasin B binding sites in the BLM was not associated by increase in the amount of intestinal GLUT2 protein. This difference was not due to differences in the extents of contamination of the BLM preparations, because there were no significant differences in the specific activities of marker enzymes for the BLM in preparations from the three groups (data not shown).

Other glucose transporter isoforms might be associated with the glucose transport activity in the BLM in rats with hyperglycemia, because the cytochalasin B binding assay measured the total number of transporters irrespective of their isoform [23]. Cheeseman et al. observed rapid regulation of the glucose transporter in BLM vesicles by [^{3}H]cytochalasin B binding assay [10–13]. Infusion of high-glucose solution for 6 h stimulated glucose transport (4-fold) and increased cytochalasin B binding to 1.8-fold that of non-infused animals. However, vesicles prepared from animals 4 h after in vivo injection of cycloheximide showed 80% reduction in glucose transport with no significant change in the cytochalasin B binding [12]. The increase in transport resulting from hyperglycemia appears to be due to increase in the functional activity of existing carriers in the membrane followed by recruitment of new carriers, as in fat cells [24]. They suggest that newly recruited carriers could have a lower K_t and a higher turnover rate.

We performed RNA-blot analyses of rat small intestine with GLUT1, -3, -4 and -5 cDNA, but could detect no signal except that for GLUT5 mRNA [8,9]. Recently, Davidson et al. showed that GLUT5 protein is located in the BBM in human small intestine [25]. We could not detect this transporter in the BLM in rat jejunum with rabbit anti-GLUT5 antibody (RaGLUT-5AP, East Acres, Biogenesis, Bournemouth, UK). Since the affinity for glucose transport and cytochalasin B binding in the BLM of rats with hyperglycemia is similar to those in rats with diabetes and those given a high-carbohydrate diet, we suggest that the functional glucose transporter in the BLM of rats with hyperglycemia may have a low affinity for glucose, like that of GLUT2.

As the changes in transport capacity in the small intestinal BLM are not nearly as marked as those in adipocytes, they may correspond to a physical response occurring shortly after the start of a meal that increases the absorption of a very important nutrient [12]. The interesting finding in the present study was that there was a mechanism for rapid regulation of glucose transport that does not depend on change in the amount of GLUT2.

REFERENCES

[1] Hopfer, U. (1987) in: Physiology of the Gastrointestinal Tract, 2nd edn. (Johnson, L.R. ed.) pp. 1499–1526, Raven Press, New York.
[2] Wright, E.M., Turk, E., Zabe, B., Mundlos, S. and Dyer, J. (1991) J. Clin. Invest. 88, 1435–1440.
[3] Bell, G.I., Kayano, T., Buse, J.B., Burant, C.F., Takeda, J., Lin, D., Fukumoto, H. and Seino, S. (1990) Diabetes Care 13, 198–208.
[4] Thorens, B., Charron, M.J. and Lodish, H.F. (1990) Diabetes Care 13, 209–218.
[5] Thorens, B., Cheng, Z.Q., Brown, D. and Lodish, H.F. (1990) Am. J. Physiol. 259, C279–C285.
[6] Cramer, C.S., Pardridge, W.M., Hirayama, B.A. and Wright, E.M. (1992) Diabetes 41, 766–770.
[7] Gould, G.W., Thomas, H.M., Jess, T.J. and Bell, G.I. (1991) Biochemistry 30, 5139–5145.
[8] Miyamoto, K., Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagihira, H. (1991) Biochem. Biophys. Res. Commun. 181, 1110–1117.
[9] Miyamoto, K., Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagihira, H. (1992) Biochem. Biophys. Res. Commun. 183, 626–631.
[10] Maenz, D.D. and Cheeseman, C.I. (1986) Biochim. Biophys. Acta 860, 277–285.
[11] Maenz, D.D. and Cheeseman, C.I. (1987) J. Membrane Biol. 97, 259–266.
[12] Cheeseman, C.I. and Maenz, D.D. (1989) Am. J. Physiol. 256, G878–G883.
[13] Cheeseman, C.I. and Harley, B. (1991) J. Physiol. 437, 563–571.
[14] Solberg, D.H. and Diamond, J.M. (1987) Am. J. Physiol. 252, G574–G584.
[15] Hopfer, U. (1990) Methods Enzymol. 192, 389–408.
[16] Scalera, V., Storelli, C., Storelli-Joss, C., Hasse, W. and Murer, H. (1980) Biochem. J. 186, 177–181.
[17] Thorens, B., Sarkar, H.K., Kaback, H.R. and Lodish, H.F. (1988) Cell 55, 281–290.
[18] Laemmli, U.K. (1970) Nature 277, 680–685.
[19] Nakabou, Y., Okita, C., Takano, Y. and Hagihira, H. (1974) J. Nutr. Sci. Vitaminol. 20, 227–234.
[20] Nakabou, Y., Ishikawa, Y., Misake, A. and Hagihira, H. (1980) Metabolism 29, 181–185.
[21] Csaky, T.Z. and Fischer, E. (1981) Diabetes 30, 568–574.
[22] Diamond, J.M. and Karasov, W.H. (1984) J. Physiol. 349, 419–440.
[23] Kasahara, M. and Hinkle, P.C. (1976) Proc. Natl. Acad. Sci. USA 73, 396–372.
[24] Suzuki, K. and Kono, T. (1980) Proc. Natl. Acad. Sci. USA 77, 2542–2545.
[25] Davidson, N.O., Hasuman, A.M., Ifkovits, C.A., Buse, J.B., Gould, G.W., Brunt, C.F. and Bell, G.I. (1992) Am. J. Physiol. 262, C795–C800.
[26] Miyamoto, K., Oka, T., Fujii, T., Yamaji, M., Minami, H., Nakabou, Y. and Hagihira, H. (1989) J. Biochem. 106, 167–171.
[27] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[28] Yen, K.H., Pan, P.C. and Holt, P.R. (1991) Gastroenterology 101, 312–318.