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Overexpression of glucose transporter modulates insulin biosynthesis in insulin producing cell line

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Glucose transporter (GT) has been suggested to be involved in the insulin biosynthesis. However, the functional relationship between GT and insulin biosynthesis is not well understood. In this report, we have generated rat pancreatic B cell lines (RINr) that stably overexpress a cDNA encoding the brain type GT. These cell lines showed 3- to 4-fold increase in insulin mRNA and protein. These results suggest that GT might have some relationship to the insulin biosynthesis in the pancreatic B cells.

Glucose transporter; Insulin biosynthesis

1. INTRODUCTION

Glucose uptake into pancreatic B cells of the islets of Langerhans, in response to the increase in glucose concentrations, is the physiological signal that induces insulin biosynthesis and release. While glucose stimulates insulin biosynthesis at the translation level in a short period of time [1], long-term modulation of insulin mRNA content by glucose has been also reported [2]. Glucose metabolism has been thought to be important for the insulin biosynthesis and glucokinase appears to be a rate-limiting enzyme in glucose metabolism in pancreatic B cells [3] because of its kinetic properties. Recently liver type glucose transporter (GT) has been isolated and demonstrated to be localized at the plasma membrane of hepatocyte and B cells in islets of Langerhans. It is speculated that in pancreatic B cells, liver type GT functions in tandem with the glucokinase as the 'glucose sensor' [4].

In the insulin producing RINr cell line, insulin secretion is not regulated in response to glucose concentrations [5]. As other secretagogues are able to stimulate insulin secretion, defects of the responsiveness to glucose in RINr might be related to defects of either glucokinase or GT. However, the relationship between GT and insulin biosynthesis is not well understood. Here we have introduced brain type GT in RINr and studied the effects on the insulin biosynthesis and secretion.

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2. MATERIALS AND METHODS

2.1. *Overexpression of glucose transporter in RINr cell line*

The pMTH-RaGT is constructed as described [6]. Transfection of RINr cells was carried out by using the calcium phosphate procedure as described [6]. The cells expressing the rabbit brain type GT were identified by Western blot analysis of the cell lysate using an antipeptide antibody against the C-terminal domain of the rabbit brain glucose transporter as described [6].

2.2. *RNA analysis*

Total cellular RNA was size-fractionated on 1% agarose-formaldehyde gels, blotted onto nylon membrane in 10×SSC (1.5 M NaCl, 0.15 M sodium citrate) and fixed under the UV light. After hybridization, filters were washed and autoradiographed.

2.3. *Western blotting analysis*

Cells were solubilized with 1% Triton X-100 in 1 mM phenylmethylsulfonylfluoride, 150 mM NaCl, 50 mM Hepes, pH 7.6, for 30 min at 4°C, followed by centrifugation at 13 000 × *g* for 30 min. The supernatants (100 μg) were subjected to SDS-polyacrylamide gel electrophoresis. Electrophoretic transfer to nitrocellulose paper and detection of the immunecomplex with [^{125}I]-labeled protein A (Amersham) were carried out as described previously [6] using antipeptide antisera against brain type glucose transporter at 1:20 dilution.

2.4. *Measurement of insulin*

Insulin levels were determined by double antibody radioimmunoassay using rat insulin as standard.

3. RESULTS AND DISCUSSION

We have investigated the consequences of introduction of rabbit brain type GT on the insulin biosynthesis and secretion in RINr cells. A 2.5 kb rabbit brain GT cDNA containing whole coding sequence was inserted downstream the metallothionein I promoter and designated pMTH-RaGT. RINr cells were transfected

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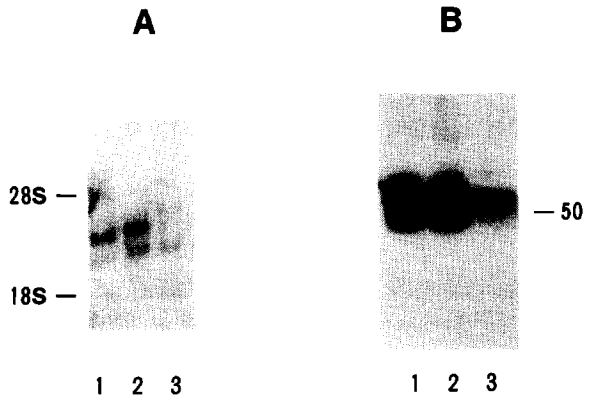

Fig. 1. Expression of brain type glucose transporter. (A) Northern blotting analysis. Twenty μg of total RNA were electrophoresed on the 1% formaldehyde agarose gel and transferred to nylon membrane. Filters were hybridized with [^{32}P]-labeled KpnI fragment of rabbit brain type glucose transporter cDNA. Lanes 1, 2: RIN/GT transfected cell line (clones 9, 12). Lane 3: control RIN/MT. (B) Western blotting analysis. One hundred μg of the proteins were electrophoresed on 10% polyacrylamide gel and transferred to nitrocellulose filters. The filters were incubated with anti-peptide antibody against brain type glucose transporter and followed with [^{125}I]-labeled protein A. Lane 1: RIN/GT9, lane 2: RIN/GT12, lane 3: control RIN/MT. 50: molecular weight marker (50 kDa).

with this recombinant DNA and cells were selected for their ability to grow in the medium supplemented with G418 (300 μg/ml). After 2 to 3 weeks in the selective medium, viable cell populations were selected.

The transcription of transfected cDNA sequences in RINr cells was tested by hybridization with rabbit brain GT cDNA probes. In addition to the endogenous mRNA (2.3 kb) another RNA band (4.1 kb) was observed (Fig. 1A, lanes 1, 2). Since the latter band was not present in RNA from vector-transfected control cell line (RIN/MT) (Fig. 1A, lane 3), we presumed that it was the product of the transfected DNA. These cell lines were designated as RIN/GT and used for further experiments. Westen blotting using a brain type GT-specific antibody demonstrated an expression of GT in RIN/GT cell lines (Fig. 1B). Its molecular weight is consistent with the expected one for the rabbit brain GT. Quantitation of the radioactivity in the GT band demonstrated that clones RIN/GT9 and RIN/GT12 contained 2.8- and 3.5-fold more GT protein, respectively (Fig. 1B, lanes 1, 2) as compared with control RIN/MT (Fig. 1B, lane 3). Expression levels of the brain GT protein correspond approximately to the amount of the transcript.

Interestingly, RIN/GT cells secreted a greater amount of insulin in the medium compared with parental cells. Parental RINr cell secreted 2.4 ± 0.2 μU/10^6 cells/h, whereas RIN/GT9 cells did 8.6 ± 1.0 μU/10^6 cells/h (Fig. 2(a)). Approximate 4 times increase in insulin secretion was observed in more than 20 independent clones. The insulin contents measured by acid extraction of the cells were 6 ± 0.3 ng in control cells and 18 ± 5 ng in mixed RIN/GT cells (per 5×10^5 cells/3 h). Theophylline (1 mM) did not seem to change insulin contents in both cells (Fig. 2(b)). To investigate whether the increased insulin production was due to translational control or not, insulin mRNA was probed with rat insulin I cDNA (a generous gift of Dr. H. Okamoto). Insulin mRNA level was increased more than 3 times in RIN/GT cells compared to that of RIN/MT cells (Fig. 3). However, no significant change was observed in the amounts of insulin mRNA in response to the increased glucose concentration (from 2 to 20 mM) of the medium in 4 h (Fig. 3) or in 24 h (data not shown).

As we have described here, overexpression of brain type GT enhances insulin mRNA content and insulin secretion in RINr cell line. Various cell lines that were transfected with the pMTH constructs which had the GT sequence in an anti-sense orientation or lacked the GT sequence did not show the increases in insulin secre-

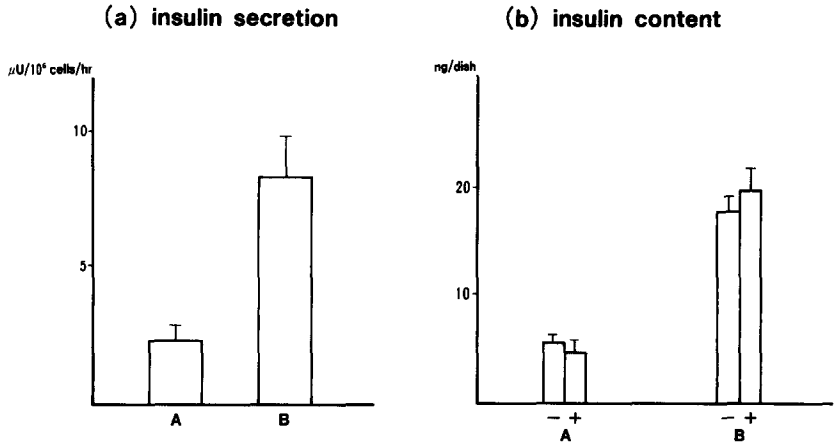

Fig. 2. Insulin production levels. Immunoreactive insulin in the medium (a) and acid extracts of the cells (b) were measured as described in Materials and Methods. (a) Insulin secretion per 10^6 cells/h. A: control RIN/MT. B: RIN/GT9. (b) Insulin content was measured in the absence (−) or presence (+) of 1 mM theophylline (5×10^5 cells/3 h/dish). A: average of 6 control lines, B: average of 8 RIN/GT cell lines.

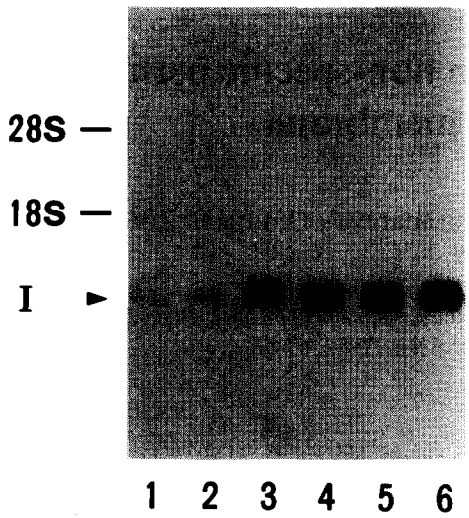

Fig. 3. Insulin mRNA levels. Ten μg of total RNA were electrophoresed and transferred to the nylon membrane. Filters were hybridized with [^{32}P]-labeled rat insulin I cDNA. I: insulin mRNA. Lanes 1–2: control RIN/MT. Lanes 3–4: RIN/GT9. Lanes 5–6: RIN/GT12. Lanes 1, 3, 5: 2 mM glucose. Lanes 2, 4, 6: 20 mM glucose.

tion or insulin mRNA (data not shown). These results suggest that the enhanced insulin biosynthesis is due to the high level of expression of GT and is not due to artifacts such as insertion mutations associated with the generation of these cell lines, although precise mechanisms are not yet known.

RINr produces insulin constitutively and its response to glucose is deteriorated. In addition to liver type GT, which normal pancreatic B cells express, RINr cell has been reported to express brain type GT, K_m of which is lower than that of liver type GT [4]. These results have raised a possibility that the additional expression of brain type GT is the cause of the unresponsiveness of RINr cells [4]. Since a change in glucose concentrations in the medium did not affect the level of insulin mRNA in RIN/GT which expressed normal brain type GT (Fig. 3), one can exclude a possibility that the brain type GT existing in parental RINr is abnormal, resulting in unresponsiveness of RINr to glucose. It is interesting to hypothesize that insulin regulation may be frozen in a high glucose state in RINr cells [7]. Regardless of the mechanisms, the results described here suggest that GT might play a role in the regulation of insulin biosynthesis. Using the strategy employed in the present study it would be of great interest to develop RINr cell lines that overexpress the liver type GT.

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