Increased Expression of a Regenerating (reg) Gene Protein in Neonatal Rat Pancreas Treated with Streptozotocin

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Abstract. We examined the expression of reg protein in neonatal rat pancreas treated with streptozotocin (STZ) by means of the immunohistochemical technique and northern blotting. Seven days after STZ injection, the plasma glucose levels in STZ-treated neonatal rats were significantly higher than those in control rats. Scattered distribution of reg protein in pancreatic islet cells was clearly observed in STZ-treated rats, but not in control rats. On the other hand, reg protein was positively stained in the exocrine cells in both groups of rats. Northern blot analyses revealed that the expression of insulin mRNA markedly decreased in STZ-treated rat pancreas, but a significant increase in reg mRNA expression was recognized in the STZ-treated rat pancreas compared with that of control rats. Rats treated with STZ during the neonatal period have been used as a model of non-insulin-dependent diabetes mellitus (NIDDM) and beta cell regeneration. Thus, the increased reg gene expression in neonatal STZ-treated rat pancreas was therefore described for the first time, and this would be a useful model for studying the relationship between NIDDM and beta cell regeneration or reg gene protein.

Key words: reg protein, Pancreatic islet cell, Neonatal rat, Streptozotocin, Beta cell regeneration

WHEN neonatal rats were treated with streptozotocin (STZ), they exhibited a transient hyperglycemia that was somewhat restored later, and ultimately developed non-insulin-dependent diabetes in the adult [1-4]. These changes were accompanied by a marked initial destruction and loss of pancreatic beta cells rapidly followed by the signs of repair. This latter process might be caused by beta cell regeneration or differentiation. A cDNA termed reg was recently isolated by differential screening of a library prepared from regenerating islets isolated from pancreatic remnants of rats subjected to 90% pancreatectomy and nicotinamide treatment [5]. We have previously reported that insulin administration to the BB/Wor+/Tky rat, a spontaneously occurring IDDM model, induced remission of diabetes and the concomitant appearance of regenerating gene protein in pancreatic islets [6]. In this study, we examined the expression of reg gene protein in neonatal rat pancreas treated with STZ, by means of the immunohistochemical technique and northern blotting. This study is the first to show the increased reg gene expression in neonatal STZ rat pancreas.

Materials and Methods

Animals

On the second day after birth, 100 mg/kg body weight of streptozotocin (STZ) dissolved in 25 µl
of citrate buffer (50 mM, pH 4.5) was intraperitoneally injected into normal Wistar rats (n=10). STZ was purchased from Sigma (St Louis, MO). Control rats received vehicle alone (n=10). On the 7th day after injection, all the rats were sacrificed for experiments.

**Biochemical study**

After anesthesia with ethyl ether, a blood sample was drawn by heart puncture and the pancreas was taken for biochemical and histological examinations. The plasma glucose level was measured by the glucoseoxidase method, applying Glutest E and chips (Sanwa Kagaku Co. Ltd. Japan).

**Histological observations**

For light microscopy, pancreatic tissue samples were immediately fixed in formalin solution and then embedded in paraffin. The sections were immunohistochemically stained either with mouse anti-rat reg protein monoclonal antibody (1:500) or guinea pig anti-swine insulin antibody (1:200), applying an LSAB Kit (DAKO Co., Ltd., Calif., USA). Counterstaining was done with Meyer’s hematoxylin as the usual method. Anti-reg protein antibody was kindly donated by Prof. H. Okamoto of the 1st Department of Biochemistry, Tohoku University School of Medicine.

**Northern blotting**

RNA from the pancreas was isolated by a guanidine thiocyanate method [7], electrophoresed on a 1.5% agarose gel, transferred onto a nitrocellulose filter, and hybridized to the 32P-labeled specific cDNA probe of rat preproinsulin or rat reg. The filters were washed and autoradiographed. Quantitation of the target RNAs was performed by scanning densitometry. The rat reg cDNA was kindly donated by Prof. H. Okamoto of the 1st Department of Biochemistry, Tohoku University School of Medicine.

**Statistical analyses**

Unpaired Student’s t-test was used for statistical analyses with 95% confidence of significance.

**Results**

Seven days after STZ-injection, the plasma glucose levels in STZ-treated rats were significantly higher than those in control rats (P<0.01, Table 1). Representative pictures of pancreatic tissues from STZ-treated and control rats are shown in Fig. 1. Islet from control rats was intact and positively stained with anti-insulin antibody, while islet from STZ-treated rat was damaged in the structure. Scattered distribution of reg protein in pancreatic islet cells was observed mainly in STZ-treated rats, but not in islets of the control rats. On the other hand, reg protein was positively stained in the exocrine cells in both groups of rats. Figure 2 shows northern blots of the pancreas RNA sample hybridized with the radiolabelled preproinsulin and reg cDNA probes, respectively. Northern blot analysis revealed that the expression of insulin mRNA markedly decreased in STZ-treated rats, but an increase in reg expression was recognized in the STZ-treated rat pancreas compared with that of control rats. When expressed as a percentage of the level found in the non-STZ treated rat, the level of reg mRNA in the STZ-treated rat was significantly increased (P<0.05, Fig. 3).

**Discussion**

Terazono et al. first reported the prominent expression of reg protein in the model of islet regeneration, using 90% pancreatectomized nicotinamide-treated rats [5]. Miyaura reported the rebound expression of reg protein in the insulinoma-bearing NEDH rat after its removal [8]. We recently reported the appearance of reg protein in the pancreatic islets in the remission BB/Wor/
In this study, we found a new model in which there was increased reg gene expression in the whole pancreas and the appearance of reg protein in the pancreatic islet cells. Neonatal rats treated with STZ exhibit an insulin-deficient acute diabetes which is characterized by spontaneous remission [1-4], in contrast with STZ-induced irreversible diabetes in adults [9].

In the neonatal STZ-induced diabetic rat, it has been shown that the recovery of the pancreatic insulin content was related to an increase in total beta cell mass [10] and that this regeneration was characterized by new islets budding from the ducts and by replication of beta cells in preexisting islets [11]. There are several reports which indicate a close relationship between reg gene expression and replication of islet beta cells in vivo and in vitro [6, 12-14]. The reg protein is, in some ways, considered to act on pancreatic beta cells as an autocrine growth factor [15].

This finding which revealed that the strong stained reg protein in STZ-treated neonatal rat pan-

Fig. 1. Representative pictures of pancreatic islets obtained by histological examinations. The pictures show the pancreases from STZ-treated (STZ+) and non-STZ treated (STZ−) Wistar rats at the 7th day after STZ-injection. Each pancreas was stained with anti-insulin antibody (Insulin) or anti-reg protein antibody (Reg) applying an LSAB kit.
creas could be new evidence of a correlation between beta cell regeneration after injury and reg protein. Recent reports showed that the reg sequence is identical to that of pancreatic stone protein [14, 16, 17] and pancreatic thread protein [18] which are expressed as pancreatic acinar cells. We could not clarify whether the increased reg gene expression was mainly derived from acinar or islet cells in this study, however, the strong stained reg protein in islet cells of STZ-treated neonatal rats suggested that the expressing reg protein was also pronounced in islet cells after injury by STZ. In situ hybridization is required to reveal the localization of reg gene expression, but a direct relationship between reg protein and beta cells remains to be clarified. This model would be able to contribute to further investigation of beta cell repair or regeneration including the possible interrelationship between exocrine and endocrine pancreas.

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References


