Compensatory Function of Submandibular Gland in Mice with Streptozotocin Diabetes under Conditions of Transplantation

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Transplantation of the submandibular gland under the renal capsule of mice with streptozotocin-induced diabetes mellitus stimulated the compensatory function of the recipient submandibular gland. An increase in insulin I and insulin II gene expression in the submandibular gland after transplantation was demonstrated by PCR. More intensive production and extrusion of these proteins in the apical and basal directions in granular compartment cells of the submandibular gland was confirmed by electron microscopy. All these changes led to a reduction of blood glucose levels in diabetic animals as soon as 2-2.5 weeks after transplantation.

Key Words: *submandibular salivary gland; streptozotocin diabetes; transplantation; insulin I; insulin II*

Insulin responsible for the maintenance of blood glucose level is produced by pancreatic β-cells in response to elevation of glucose level. Autoimmune type I diabetes is associated with destruction of β-cells and disorders in insulin secretion leading to chronic hyperglycemia. Insulin insufficiency causes metabolic disorders; the patients develop such threatening complications as glycosuria, polyuria, angiopathy, *etc.* Life-long insulin therapy remains the main method for the treatment of patients with diabetes mellitus. Numerous studies are now focused on the use and creation of sources of insulin-producing cells. The potentialities of transplantation of the pancreas, embryonic stem cells, and multipotent bone marrow cells for correction of insulin and glucose metabolism in diabetics have been studied [4,6,8,9,13-15]. One more pressing problem is blood glucose level fluctuations and emergence of the so-called peaks. The search for new means providing stable compensation of diabetes mellitus and reducing the risk of secondary complications is an important problem of modern science. The use of salivary glands as the source of insulin-producing cells seems to be a promising approach: numerous experimental data demonstrated the presence of insulin-like protein in cells of the granular compartment of submandibular gland (SMG) ducts [3,10,11]. This protein is similar to pancreatic insulin by many characteristics in this animal species [3].

The insulinogenic function of the salivary glands as the factor playing an accessory compensatory role under conditions of insufficient insulin production has been discussed for many years. Maternal hyperglycemia (diabetes mellitus) leads to compensatory reactions of fetal pancreatic insular system: restructuring of the pancreatic organogenesis towards an increase in the counts and size of β-cells and insulin production. This restructuring often leads to exhaustion of β-cell functional activity and hence, to the development of islet destruction and insulinemia in the progeny. SMG organogenesis can change under these conditions and its compensatory effects can manifest at an earlier age [2]. In addition, the compensatory function of the salivary gland has been found in depancreatized animals, in animals with induced diabetes mellitus, and animals treated with isoproteronol (nonselective β-adrenergic receptor agonist) [1,3]. It is noteworthy that isoproteronol treatment stimulates the synthesis and extrusion of insulin-like protein into the blood, which leads to reduction and even disappearance of diabetes symptoms in animals [1,3].

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We studied the synthesis and extrusion of insulin under conditions of transplantation of SMG from normal animals to mice with streptozotocin (STZ)-induced diabetes mellitus, the most prevalent model of diabetes mellitus. We also evaluated the effect of SMG transplantation on glycemia level in diabetic animals.

MATERIALS AND METHODS

SMG of male C57Bl/6 mice (20-22 g) were used in the study. Hyperglycemia was induced by single intraperitoneal injections of STZ (40 mg/kg) for 5 days. Blood sugar level was measured by the standard One Touch Ultra glucometer (Johnson&Johnson). Stable hyperglycemia (above 350 mg/dl glucose) developed 5-6 days after STZ injection. Donor tissue (14-15 mg) from the ductal part of SMG from normal animals was transplanted under the renal capsule to anesthetized males in order to attain the expected stimulation of insulin synthesis in SMG. Since the level of lethal factor in SMG from adult male mice is higher than in female SMG, transplantation of SMG from females to males causes no animal death in the overwhelming majority of cases. Therefore, the females served as SMG donors in our study. Measurements of blood glucose were carried out over 2 weeks before transplantation and every other day throughout 8.5 weeks after transplantation. Intact animals and animals with STZ diabetes served as controls. For microscopic studies of SMG, the material was fixed in Carnoy's fluid, embedded in paraffin, and the sections were stained with hematoxylin and eosin. For electron microscopy, tissue specimens were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, dehydrated, and embedded in epon by the standard method. Ultrathin sections were contrasted with lead citrate and examined under a Jeol-100B microscope. Total RNA for PCR analysis was isolated using TRI® Reagent (Sigma). The mRNA fraction was isolated from total RNA on columns according to manufacturer's instruction (Sigma). cDNA was constructed on mRNA using M-MLV reverse transcriptase, hexanucleotides, and oligo-$d(T)_{18}$ primer (Silex M). The primers for PCR were constructed using DNAStar software. The nucleotide sequences and lengths of PCR fragments are presented in Table 1. PCR with specific primers was carried out on a cDNA template using Colored-Taq-polymerase (Silex M) on a Mastercycle amplifier (Eppendorf). Amplification conditions were as follows: 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, 40 cycles. Gene expression was evaluated by fluorescence intensity of bands obtained after electrophoretic separation of PCR products in 1.5% agarose gel with ethidium bromide on a gel analyzer (Bio-Rad) using Quantity One® software for analysis of electrophoretic images (Bio-Rad). For quantitative evaluation of the expression of the studied genes, cDNA were pre-standardized by RPL-19 gene. Amplification conditions were as follows: 1 min at 94°C, 45 sec at 58°C, 1 min at 72°C, 30 cycles. cDNA were assumed to be standardized if the level of GAPDH expression in the libraries was the same. The data were statistically processed by MS Excel software. The means and standard deviation were evaluated. The significance of differences was evaluated by Student's *t* test. The differences were considered significant at $p<0.05$.

TABLE 1. Nucleotide Sequences of Primers for PCR Analysis

Gene	Primer nucleotide sequence	Length of PCR fragment, b.p.
Insulin I	5' atggccctgtggatgcgctt 3'	249
	5' tagttgcagtagttctccagct 3'	
Insulin II	5' atggccctgtggatccgctt 3'	331
	5' tagttgcagtagttctccagct 3'	
RPL-19	5' agggtactgccaatgctcgga 3'	326
	5' ccttgacagagtcttgatgatc 3'	

RESULTS

Diabetes is associated with morphological and functional changes, including changes in the expression of secretory proteins, accumulation of lipid droplets in secretory cells, reduced response to cholinergic stimulation, increase of autophagal and lysosomal activities, endocytosis of secretory proteins, and accumulation of membrane material in ductal cells of the salivary gland [1,3,7,12]. Our experiments on animals with STZ diabetes confirmed the development of some of these changes.

In animals (n=8) with stable diabetic status (blood glucose concentration >350 mg/dl, urinary glucose 112 mg, ketones >2; polyuria, body weight loss), reduction of blood glucose level was observed 2-2.5 weeks after transplantation of normal SMG tissue under the renal capsule. During the next 8-9 weeks glycemia level stabilized, no sugar was present in the urine (Fig. 1), while animals with STZ diabetes without transplantation (n=5) died after 20 days.

Fixation of SMG in experimental animals led to hypertrophy of these glands. Light microscopy revealed a trend to enlargement of acinar cell areas, while the areas of SMG granular compartment cells in recipients virtually did not change. Ultrastructural studies of granular duct cells showed significant extension of the perinuclear spaces. Secretory granules often fused, many small granules appeared. The apical

parts of the majority of granular cells were swollen and detached into the duct lumen together with secretions. Mature secretory products and even dilated cisterns of the granular endoplasmic reticulum were often found in the lumens. Some cells were often partially or completely destroyed during this process. Apocrine secretion was paralleled by the formation of small vesicles and release of secretion in the basal direction (Fig. 2). This vesicular release of secretion by the endocrine type as a sign of secretion intensification had been described previously in diabetic animals treated with isoproteronol and in animals after pancreas removal [1,3]. Presumably, transplantation of SMG to animals with STZ diabetes led to intense production and extrusion of protein from the granular compartment cells. This insulin-like protein of extrapancreatic origin is sufficiently well studied [3].

The effect of isoproteronol on protein production rhythm in normal animals and animals with alloxan diabetes has been described. Protein secretion in animals with STZ diabetes is more rapidly stimulated than in normal animals [1,2]. Insulin I and II transcripts were found in SMG of recipient animals 5 weeks after transplantation under the renal capsule.

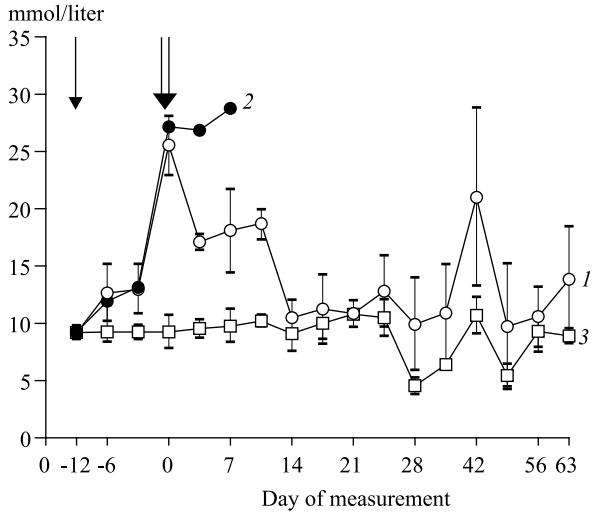

Fig. 1. Blood glucose level in C57Bl/6 mice. *1*) intact animals (control group 1); *2*) animals with STZ diabetes without transplantation (control group 2); *3*) animals with STZ diabetes after SMG transplantation. Arrow: STZ injection, 40 mg/kg; double arrow: SMG transplantation.

It is noteworthy that normally genes encoding insulin are expressed in SMG of healthy animals. The majority of mammals express one insulin gene organized

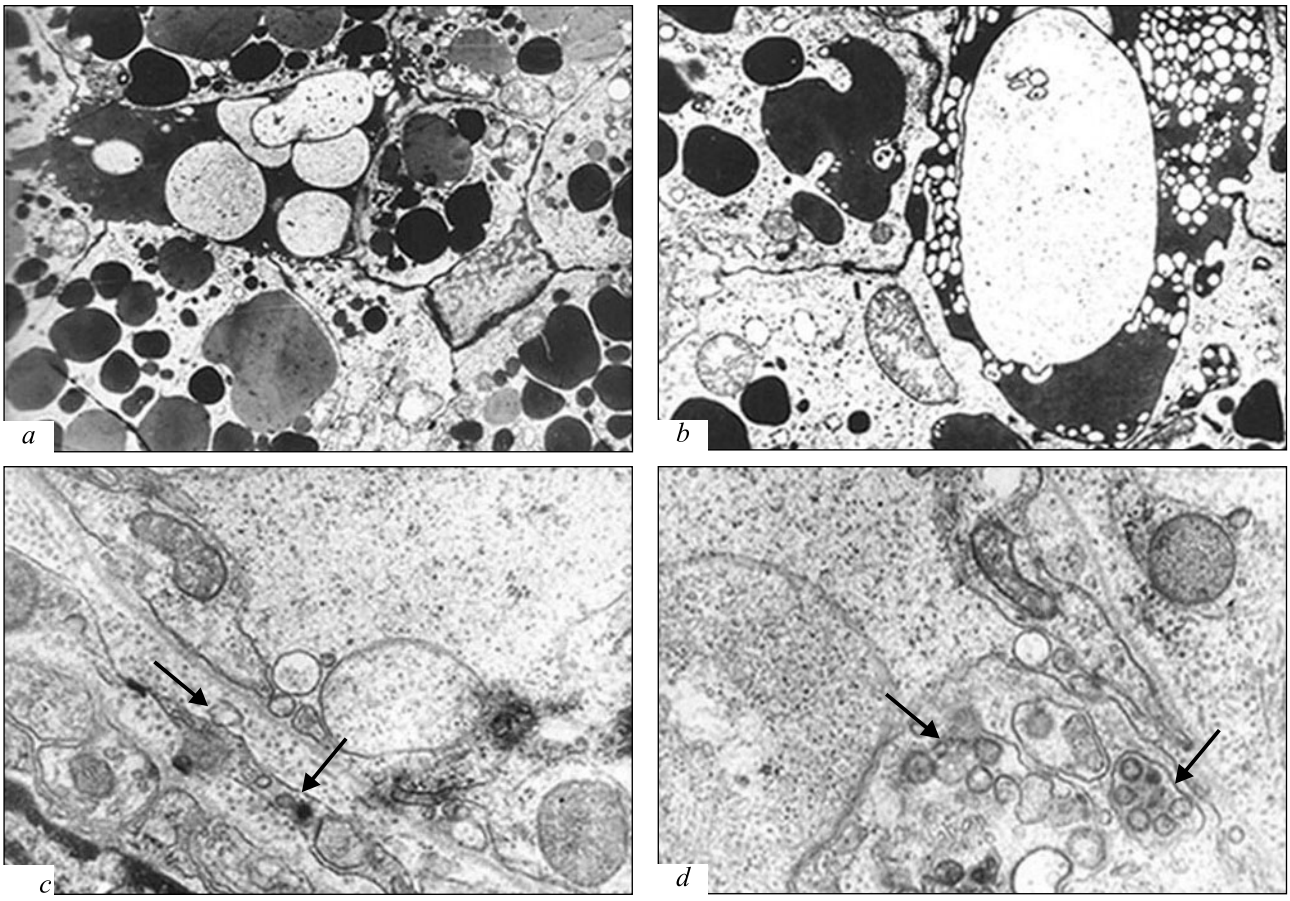

Fig. 2. Secretion release in the mouse SMG granular compartment cells. *a*, *b*) secretion release in apical direction, ×6.6; *c*, *d*) small vesicles in interstitium (arrows), ×40.

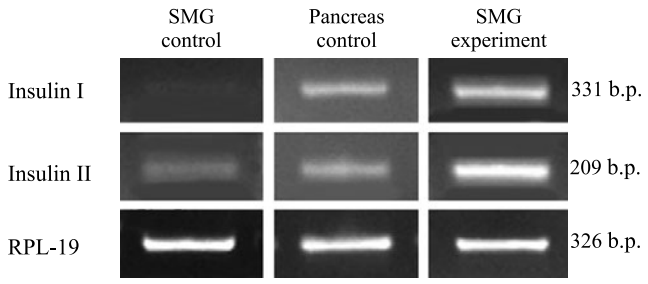

Fig. 3. PCR analysis of the expression of insulin I and insulin II genes in SMG and pancreas of intact animals and in SMG of recipients. cDNA standardized by ribosomal protein (RPL-19).

like the human gene. In rats and mice, two nonallele genes encode proinsulin giving rise to two different active insulin molecules. The expression of the studied genes in mice with STZ diabetes after transplantation was significantly higher than in controls (Fig. 3). It should be emphasized that the expression of insulin I and II in SMG was more pronounced than in the pancreas of normal animals.

Thus, transplantation of SMG under the renal capsule of diabetic animals stimulated production and accumulation of insulin in cells of SMG granular ducts of mice and extrusion of insulin, which was confirmed by electron microscopy. The stimulatory effect of transplanted SMG manifested in transformation of recipient's SMG into an extra source of insulin-producing cells. The decrease in glycemia level and the above changes in insulin expression in experimental animals suggest the transplantation stimulation of insulin synthesis and confirm previously described compensatory function of SMG [1,3].

Further studies of the compensatory function of the SMG under conditions of allogenic transplantation seem to be of theoretical and practical importance, as an approach to the search for new sources of insulin-producing cells for the treatment of diabetes mellitus.

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