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Impaired Insulin Secretion in the Spontaneous Diabetes Rats

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KIMURA, K., TOYOTA, T., KAKIZAKI, M., KUDO, M., TAKEBE, K. and GOTO, Y. *Impaired Insulin Secretion in the Spontaneous Diabetes Rats.* Tohoku J. exp. Med., 1982, **137** (4), 453–459 —— Dynamics of insulin and glucagon secretion were investigated by using a new model of spontaneous diabetes rats produced by the repetition of selective breeding in our laboratories. The perfusion experiments of the pancreas showed that the early phase of insulin secretion to continuous stimulation with glucose was specifically impaired, although the response of the early phase to arginine was preserved. The glucose-induced insulin secretion in the nineth generation (F_8) which had a more remarkably impaired glucose tolerance was more reduced than in the sixth generation (F_5). No significant difference of glucagon secretion in response to arginine or norepinephrine was noted between the diabetes rats and control ones. The present data indicate that the defective insulin secretion is a primary derangement in a diabetic state of the spontaneous diabetes rats. This defect in the early phase of glucose-induced insulin secretion suggests the specific impairment of the recognition of glucose by the pancreatic β-cells. The spontaneous diabetes rats are very useful as a model of a disease for investigating pathophysiology of non-insulin dependent diabetes mellitus. —— insulin; glucagon; spontaneous diabetes rats

Experimental diabetes animals have contributed to the progress of studies on pathogenesis of diabetes mellitus. Especially, spontaneous diabetes animals have provided us with much information. A type of spontaneous diabetes rats which we used in this study had been produced in our laboratories (Goto et al. 1975; Goto and Kakizaki 1981). Briefly, these rats had been selected from normal rats showing the upper limit of normal blood glucose, and the male and female rats with abnormal glucose tolerance were selected from their offsprings and mated each other. The repetition of this procedure resulted in producing diabetic rats at the sixth generation (F_5).

They are characterized by the lack of insulin response to glucose stimulus and by an amount of secretory granules of insulin no less than the normal, which suggests an impaired secretory process of insulin (Goto et al. 1977). They also have characteristic features of diabetic complications such as thickening of the

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glomerular basement membrane (Yagihashi et al. 1978) and low nerve conduction velocity (Yamada and Kakizaki 1979). In order to elucidate the functional property of the endocrine pancreas of this model, we investigated the dynamics of insulin and glucagon secretion by using the perfusion technique of the isolated pancreas.

Materials and Methods

Diabetic rats at six months of age (F_5, F_8 and F_9) were selected with a glucose tolerance test. After an overnight fast, glucose solution (2 g per kg body weight) was orally given through a metal catheter into the stomach. Blood specimens for the determination of glucose were obtained from tail vessels before and 30, 60, 90 and 120 min after glucose ingestion.

The rats showing diabetic glucose tolerance were used for the perfusion experiments. Under intraperitoneal nembutal anesthesia (50 mg per kg body weight), the pancreas was isolated by the procedure of Grodsky et al. (1963) with some modifications (Toyota et al. 1975). The isolated pancreas was placed in an incubator. A fixed cannula was inserted into the coeliac artery for allowing perfusate and another cannula was inserted into the portal vein for collecting effluent. Perfusate was not recycled through the pancreas so as to avoid the interference of gut hormones with the function of the pancreatic islets. The perfusate used in all experiments was Krebs-Ringer bicarbonate buffer containing 4.5% dextran (T-70, Pharmacia, Sweden) and 0.2% bovine serum albumin (Fraction V, Sigma Chemical Co., USA). The perfusate was equilibrated with a mixture of 95% O_2 and 5% CO_2 for 20 min, resulting in pH 7.40. The flow rate was maintained constant at 2 ml per min. Portal effluents were collected into the polyethylene tube containing 500 KIU aprotinin and this mixture and the remaining effluent for insulin determination were frozen and stored at –20°C until assayed. The perfusion study of the diabetic rats which had been matched in body weight and age of normal ones was performed after a 15-min equilibration period with the basal perfusion medium containing 2.8 mM glucose. After epuilibration, the concentration of glucose was changed from 2.8 to 16.7 mM. The duration of stimulation by 16.7 mM glucose was 60 min in F_5 and 40 min in F_8. The effluent was collected every minute for 10 min and thereafter done every minute at 5-min intervals. For evaluating both insulin and glucagon secretion simultaneously, we performed the following experiments: 1) The pancreas was perfused with 8.3 mM glucose plus 10 mM arginine for 10 min and thereafter only with 8.3 mM glucose. 2) The pancreas was perfused with 11.1 mM glucose for 20 min and after the completion of the glucose infusion it was perfused with norepinephrine (0.5 μg/ml) for 10 min. The effluent was collected every minute for 10 min and thereafter every minute at 5-min intervals as in the previous experiments.

The concentration of immunoreactive insulin was measured by the double antibody system (Morgan and Lazarow 1962) and rat insulin was used as a standard. Glucagon was assayed by radioimmunoassay using the dextran-coated charcoal separation technique and pork glucagon was used as standard (Toyota et al. 1975). Antibody to glucagon was provided by courtesy of Dr. Koga, Shimizu Pharmaceutical Co., Japan, which reacted immunologically to enteroglucagon. ^{125}I-glucagon was purchased from Hoechst Pharmaceutical Co., Germany. Rat insulin and pork glucagon were gifts from Novo Research Institute, Denmark. Statistical evaluation was done by Student's t-test.

Results

As shown in Figs. 1 and 2, the continuous infusion of 16.7 mM glucose provoked a characteristic biphasic response of insulin secretion in normal rats. In the diabetes rats an immediate early phase of secretion was not detectable and the

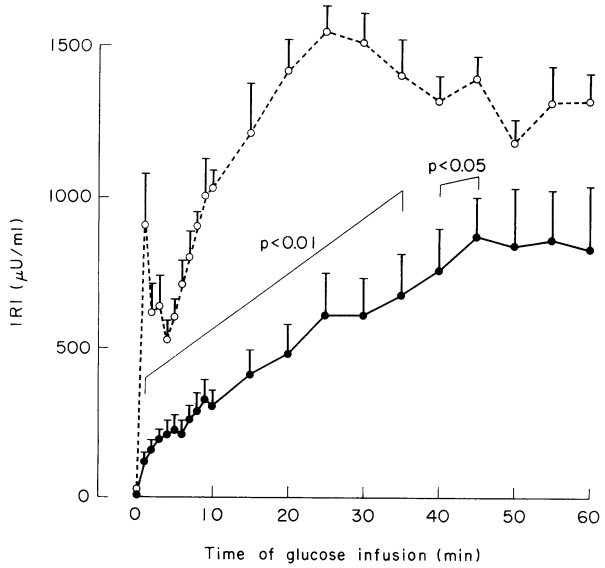

Fig. 1. Glucose-induced insulin secretion from the perfused pancreas of selectively imbred spontaneous diabetes rats (SSDR) in the sixth generation (F_5). 16.7 mM glucose was continuously infused. ○ ○, control (n=5); ●—●, SSDR-F_5 (n=6). Mean±S.E.

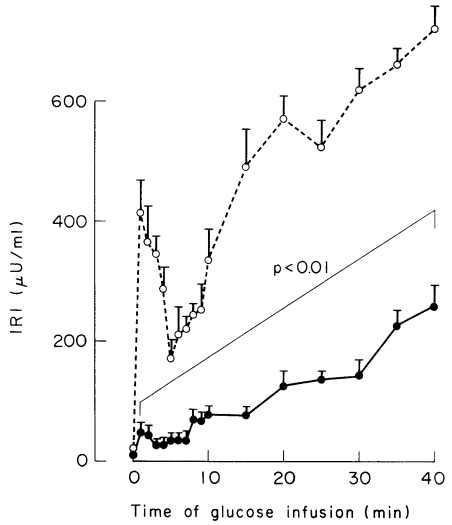

Fig. 2. Glucose induced insulin secretion from the perfused pancreas of selectively imbred spontaneous diabetes rats (SSDR) in the nineth generation (F_8). 16.7 mM glucose was continuously infused. ○ ○, control (n=5); ●—●, SSDR-F_8 (n=6). Mean±S.E.

second phase occurred very slowly throughout the 40-min stimulation period. An amount of insulin in the effluent from the pancreas of the diabetes rats (F_5 and F_8) was less than 10 per cent of normal rats. In comparison with each other, the F_8

diabetic rats showed an amount of insulin output significantly less than F_5 diabetic rats. This result corresponds to the fact that the glucose intolerance of the F_8 rats is more remarkable than that of the F_5 rats.

The F_8 diabetic rats showed a biphasic response of insulin to arginine (10 mM) infusion with glucose (8.3 mM). Simultaneously, an immediate early phase of glucagon was detectable in the diabetes rats as shown in Fig. 3. The amount of glucagon in the diabetic rats was not different from that in the normal rats.

For evaluating glucagon secretion, we carried out the final experiments showing that glucagon secretion by norepinephrine infusion in the diabetic rats was not different from that in the normal rats (Fig. 4).

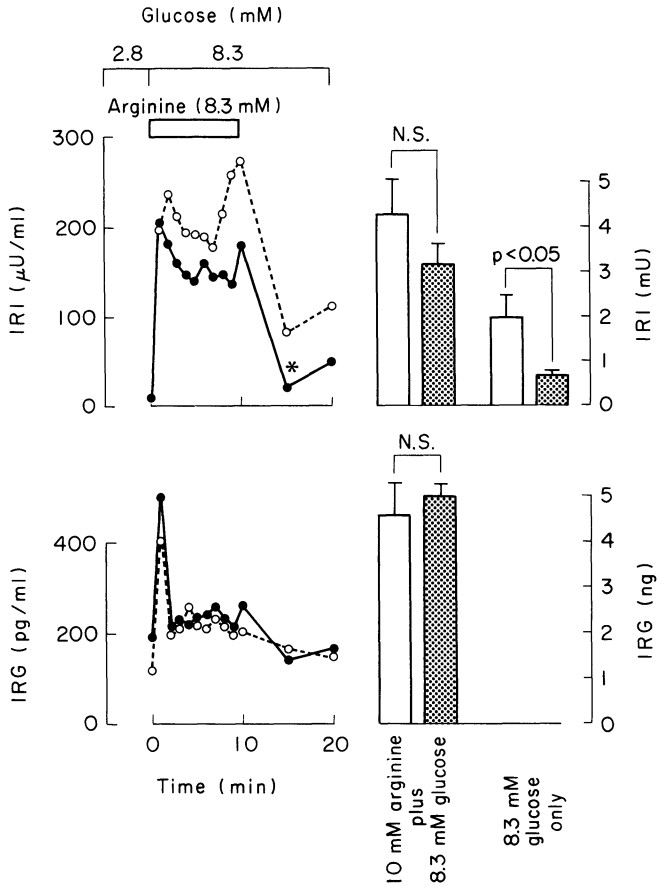

Fig. 3. Secretion of insulin and glucagon in response to 10 mM arginine plus 8.3 mM glucose or 8.3 mM glucose alone from the perfused pancreas of selectively imbred spontaneous diabetes rats (SSDR) in the nineth generation (F_8). Cumulative output of insulin and glucagon is shown in the right panel. An asterisk (*) indicates statistical significance ($p<0.05$). ○ ○, ☐, control ($n=5$); ●—●, ▨ SSDR-F_8 ($n=5$).

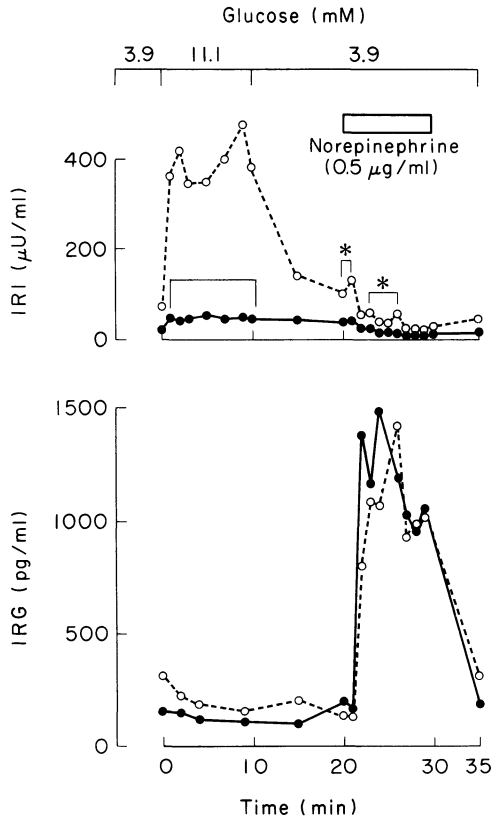

Fig. 4. Secretion of insulin and glucagon in response to 11.1 mM glucose and norepinephrine from the perfused pancreas of selectively imbred spontaneous diabetes rats (SSDR) in the tenth generation (F_9). Statistical significance * $p<0.05$, † $p<0.01$. ○ ○, control ($n=4$); ●—● SSDR ($n=4$).

Discussion

When the pancreatic islets, the β-cells, are damaged by viral infection, immune reaction or chemicals such as alloxan or streptozotocin, a diabetic state is caused as the result of the absolute lack of insulin. This type is corresponding to insulin-dependent diabetes in human.

Our present study indicates that in the spontaneous diabetes rats made by Goto et al. (1975), the first phase of glucose-induced insulin secretion is almost negligible in spite of the fact that arginine infusion provokes immediately the early phase secretion. The feature of insulin secretion of this model rat resembles that of the early stage of human diabetes. It has been well established that in maturity onset diabetes, insulin response to glucose stimulus showed characteristically reduced initial insulin release (Seltzer et al. 1967; Luft and Efendic 1979). As reported by Efendic et al. (1974), the early phase of insulin secretion to continuous

glucose infusion is characteristically impaired in the early stage of maturity onset diabetes. It is of interest to note that this defective insulin secretion is specific to glucose stimulus, but the β-cell of the pancreas in the early stage of noninsulin-dependent diabetics still responds to insulin secretagogues other than glucose such as arginine (Palmer et al. 1976), tolbutamide (Versano-Ahron et al. 1970), glucagon (Simpson et al. 1968) and isoproterenol (Halten and Porte 1978). Thus, the spontaneous diabetes rats are very similar to noninsulin-dependent diabetes in human. Because of the decrease of the recognition of glucose by the β-cell might be most responsible for this abnormality.

A question arises why insulin secretion is disturbed specifically to glucose stimulus in diabetes mellitus. As to the mechanism of the biphasic pattern of insulin secretion, two hypotheses for explanation have been now proposed; the one is "two compartments model" of Grodsky (1972), and the other is "multiplicative model" of Cerasi (1975). According to the latter model, it is assumed that the formation of early phase of glucose-induced insulin secretion is predominantly defined by the initiation of glucose recognition. In the process by which glucose stimulates insulin release, three major steps are now identified. The first is recognition of glucose on the surface of pancreatic β-cell; the second is intracellular transmission of the recognized information; the third is the effector system which induces the migration and the exocytotic extrusion of secretory granules. The last step is thought to be common pathway, whatever secretagogues might be recognized (Malaisse 1973). For this reason, the defective recognition of glucose on the pancreatic β-cell is a possible cause of specifically impaired insulin secretion in the diabetic rats. The present work demonstrated that glucagon secretion to arginine or norepinephrine stimuli was normal.

Finally, because clinical features of the diabetes rats are considerably similar to those of human noninsulin-dependent diabetes, they appear to be a highly useful model of diabetes mellitus for investigations such as on genetic analysis, metabolic and endocrine properties and the pathophysiology of the complications.

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