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ORIGINAL ARTICLE

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Glucagon attenuates the action of insulin on glucose output in the liver of the Goto-Kakizaki rat perfused in situ

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Abstract The effects of glucagon and insulin on glucose production were explored directly using the isolated perfused liver of the Goto-Kakizaki (GK) rat, an animal model of type-2 diabetes. In the perfused liver of control rats, infusion of glucagon (0.06–1.0 nM) into the portal vein dose-dependently increased glucose output. In the GK rat liver, in which the intracellular distribution of glycogen was heterogeneous, basal glucose output during perfusion was significantly higher than in control, whereas the effect of glucagon on the maximum glucose output was not different. Infusion of insulin inhibited the glucagon-induced hepatic glucose output by 30–40% in control livers, but had no effect on that from the GK rat liver. The increase in hepatic cAMP content after glucagon infusion was antagonized by insulin in control livers, but not in the livers of GK rats. These results indicate that the antagonistic effect of insulin on glucagon-induced hepatic glucose production was attenuated in the isolated liver of the GK rat and suggest that this insulin resistance appeared in the signal transduction process of glucagon upstream from cAMP production.

Keywords Type-2 diabetes · Perfused liver · Glucose production · Glycogen · Insulin · Glucagon

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM, type-2 diabetes) is considered to be associated with a relative defect of insulin secretion and insulin resistance. Insulin resistance in peripheral tissues, such as adipose tissue and skeletal muscle, decreases glucose utilization and increases blood glucose levels. However, not only the decrease in peripheral glucose uptake but also the changes of glucose production in the liver may play an important role in the hyperglycaemia in diabetes. The Goto-Kakizaki (GK) rat is a spontaneous model of type-2 diabetes that has characterized by Goto et al. [3, 4] and is used widely in the analysis of the mechanism of type-2 diabetes. This animal is characterized by impaired glucose-induced insulin secretion, decreased beta cell mass, hepatic glucose overproduction and moderate insulin resistance in muscles and adipose tissues. Although dysfunction of the pancre atic islets seems to be important [12, 14], impairment of liver function may also be involved in development of diabetes in the GK rat. However, insulin resistance in the liver has not yet been completely elucidated. Previous studies have shown that hepatic glucose production is increased during a euglycaemic glucose-clamp in vivo [2, 13]. In addition, because the affinity and the kinase activity of insulin receptors do not seem to be altered in the GK rat liver, hepatic insulin resistance in this rat is accounted for mainly by post-receptor defects, though the receptor number is decreased by 20–30% [2].

To explore insulin resistance in the liver directly, we used the isolated, perfused GK rat liver, and examined the antagonism of insulin on glucagon-induced hepatic glucose production.

Materials and methods

Liver perfusion

Diabetic male GK rats were obtained from our local colony with progenitors issued from the original colony established by Goto et al. [3, 4]. Male GK rats weighing 180–220 g were kept on a 12-h light-dark cycle (lights on 6 a.m. to 6 p.m.) with free access to water and laboratory chow (MF, Oriental Yeast, Tokyo, Japan). All experiments were started between 11 a.m. and 12 noon. Male Wistar rats (Clea Japan, Osaka, Japan) were used as non-diabetic controls of the same genetic origin. The experimental protocol was approved by the Animal Studies Committee of Ehime University.

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Livers were perfused in situ without recirculation in a 37 °C cabinet via the portal vein using Krebs-Henseleit bicarbonate buffer as previously reported [8], except that the buffer did not contain glucose, lactate or pyruvate. The medium was equilibrated with 95% O_2 and 5% CO_2. The perfusion pressure was constant at about 10 cm H_2O with a flow rate of 3.7–4.2 ml/min/g liver under basal conditions. The flow rate was measured by fractionating the effluent. Glucagon and insulin (Sigma, St. Louis, Mo., USA) were first dissolved in 0.1 M HCl at 10 or 100 nM, respectively, and diluted into the perfusion medium to the final concentration for infusion.

Measurement of glucose output and tissue cAMP content

To measure the plasma concentrations of glucose and lactate, blood samples were taken from the inferior caval vein before perfusion. Glucose and lactate were measured by a colourimetric method using glucose oxidase (Blood Sugar-GOD-PAP; Roche Diagnostics, Mannheim, Germany) and lactate oxidase (Determiner LA; Kyowa Medics, Tokyo, Japan). Tissue glycogen was determined using the method described previously [5]. Insulin was assayed by ELISA (Morinaga Biolaboratory Institute, Yokohama, Japan). To measure the tissue cAMP content, the liver was sampled during perfusion using a freeze-cramp technique 3 min after the onset of glucagon infusion. Tissue cAMP was assayed using the Biotrak EIA system (Amersham Pharmacia Biotech, Piscataway N.J., USA) after homogenization and extraction as described previously [15].

Electron microscopy of the liver

Tissue samples from control and GK rat livers were cut into small pieces, fixed first in 3% glutaraldehyde (0.1 M phosphate buffer, pH7.4) for 2 h at 4 °C and then in 2% osmium tetroxide (in the same buffer, 2 h, 4 °C), dehydrated in a graded ethanol series and embedded in Epon 812. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (H-800, Hitachi, Tokyo, Japan).

Statistical analysis

Values are expressed as means±SEM. Data were analysed using two-way analysis of variance followed by Newman-Keul's test for multiple comparisons. $P<0.05$ was regarded as significant.

Table 1 Body weight and liver weight in control and Goto-Kakizaki (*GK*) rats. Means±SEM, *n*=5–7 rats per group

	Body weight (g)	Liver weight (g)	Liver weight/ body weight (%)
Control	222.7±16.3	9.61±0.68	4.32±0.16
GK	242.6±16.3	9.37±0.69	3.89±0.40*

*$P<0.05$ vs. control

Results

Liver weight and hepatic glycogen content in the GK rat

Because the liver weight in GK rats was lower than in Wistar rats at the same age (data not shown), we used 7-week-old Wistar rats as a control to adjust the liver weight for perfusion experiments (Table 1). In the GK rat, the liver weight/body weight ratio was significantly lower than in controls (Table 1). Plasma glucose level in GK rats was increased approximately twofold, while plasma insulin concentration was not significantly different (Table 2). Hepatic glycogen content in GK rats tended to be higher than in control, although the difference was not significant (Table 2).

Glucagon-induced glucose output in the perfused GK rat liver

Figure 1 shows the glucagon-induced glucose output in perfused control and GK rat livers. Basal glucose output was 0.87±0.20 and 1.25±0.33 µmol/min/g liver in con-

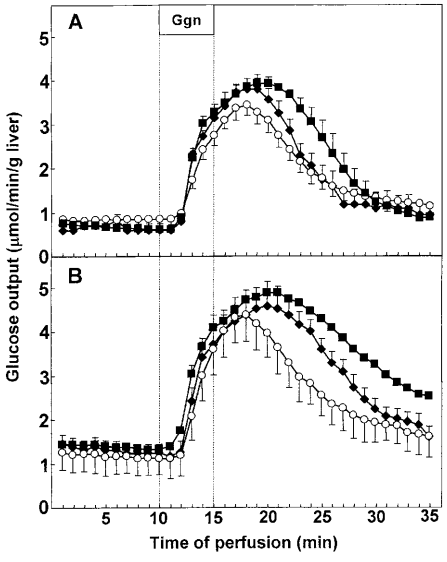

Fig. 1 Effect of glucagon (*Ggn*) on glucose output from the rat liver [**A** control; **B** Goto-Kakizaki (GK) rat] perfused in situ without recirculation in a 37 °C cabinet via the portal vein using Krebs-Henseleit bicarbonate buffer equilibrated with 95% O_2 and 5% CO_2. The perfusion pressure was constant at about 10 cm H_2O with a flow rate of 3.5–4.2 ml/min/g liver under basal conditions. Glucagon was infused at 0.06 (○), 0.2 (◆) or 1.0 (■) nM from minute 10 to minute 15 of the perfusion period. Means±SEM, *n*=4–5 rats per group

Table 2 Concentrations of plasma glucose, lactate and insulin and hepatic glycogen content in control and GK rats. Means±SEM, *n*=5–7 rats per group

	Plasma glucose (mg/dl)	Plasma lactate (mg/dl)	Plasma insulin (pg/ml)	Hepatic glycogen (mg/g)
Control	197.4±9.6	16.6±4.5	598.0±106.7	47.6±13.0
GK	328.8±63.5*	15.9±4.8	589.5±109.1	61.5±12.1

*$P<0.01$ vs. control

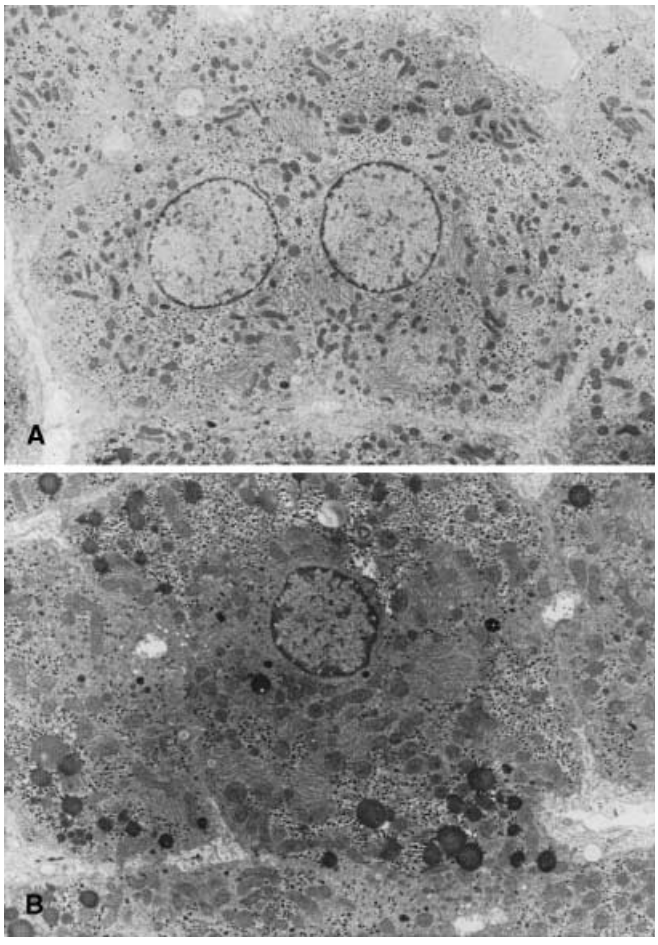

Fig. 2 Electron micrographs of liver from control (**A**) and GK (**B**) rats. Magnification ×6000

trol and GK rats, respectively (P<0.05). Infusion of 0.06 nM glucagon into the portal vein for 5 min increased hepatic glucose output up to 3.8 µmol/min/g liver in control and to 4.7 µmol/min/g liver in GK rats. Glucagon dose-dependently increased glucose output up to 1.0 nM in both control and GK rats (Fig. 1) whereby the maximum increase in glucose output from basal level elicited by glucagon was not significantly different between the two groups. This effect of glucagon on glucose output was almost totally abolished after 24 h starvation both in control and GK rats (data not shown).

Intracellular distribution of glycogen in the GK rat liver

Electron micrographs show glycogen particles in GK rat hepatocyte to be localized mainly near the plasma membrane (Fig. 2B), while those in control hepatocytes are distributed homogeneously (Fig. 2A). Accumulations of lipid droplets can also be seen in GK hepatocytes (Fig. 2B).

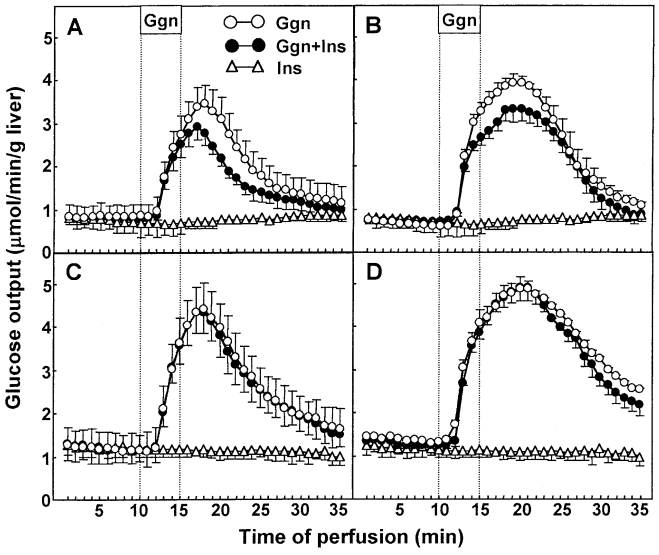

Fig. 3A–D Effects of glucagon (*Ggn*) and insulin (*Ins*) on glucose output from the perfused rat liver of control rat. Control (**A**, **B**) and GK (**C**, **D**) rat livers were perfused as in Fig. 1. Glucagon was infused at 0.06 (**A**, **C**) or 1.0 nM (**B**, **D**) from minute 10 to minute 15 in the perfusion period. Insulin was infused at 0.3 (**A**, **C**) or 100 nM (**B**, **D**) from minute 5 to minute 15 in the perfusion period. Means±SEM, n=5–7 rats per group (○ glucagon, ● Glucagon plus Insulin, Δ Insulin)

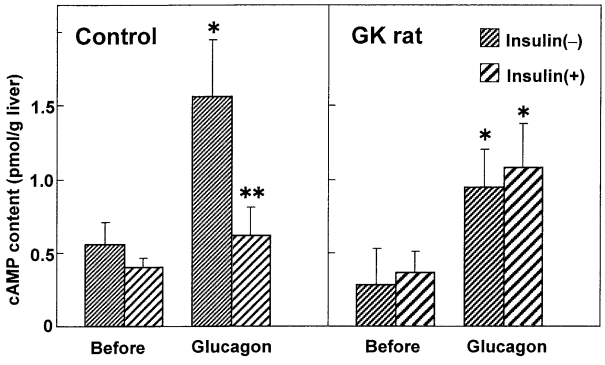

Fig. 4 Effect of insulin on the increase in hepatic cAMP content induced by glucagon in the perfused liver. Rat livers (*left*, control; *right* GK rat) were perfused and glucagon and insulin infused as in Fig. 3 at 0.06 and 0.3 nM, respectively. Tissue samples were taken by freeze-cramp technique before (*Before*) and 3 min after the onset of glucagon infusion (*Glucagon*) from the same liver. *P<0.05 vs. Before; **P<0.05 vs. glucagon alone i.e. without insulin

Effect of insulin on glucagon-induced glucose output in GK rats

In control rats, infusion of 0.3 nM insulin beginning 5 min before the onset of glucagon (0.06 nM) infusion significantly attenuated (40%, P<0.05) the increase in glucose output elicited by the latter (Fig. 3A). The increase in glucose output caused by 1.0 nM glucagon was also inhibited partially by 100 nM insulin (ca. 30%, P<0.05; Fig. 3B). In GK rats, in contrast, the effect of glucagon was not inhibited by the same concentration of insulin (Fig. 3C and D). Insulin infusion alone (0.3 or

100 nM) did not affect glucose output in either group (Fig. 3A–D).

Glucagon-induced changes in tissue cAMP content in GK rats

In the perfused liver, infusion of 0.06 nM glucagon into the portal vein increased tissue cAMP content both in control and GK rats (Fig. 4). Tissue cAMP content before and during glucagon infusion was not significantly different between control and GK rats (Fig. 4). Insulin (0.3 nM) inhibited the increase in tissue cAMP content induced by glucagon in control rats, but not in GK rats (Fig. 4). Repeated sampling of tissue from the same perfused liver without glucagon did not affect hepatic cAMP content (data not shown).

Discussion

The present study indicated that the basal output of glucose from the isolated perfused GK rat liver, i.e. in the absence of humoral factors, was increased. The heterogeneous intracellular distribution of hepatic glycogen may be involved in the enhanced output of basal glucose production in GK rats. Our results also indicated that the liver of GK rats was resistant to insulin in respect of the latter's antagonism of glucagon-induced glucose production. The insulin resistance appeared in the signal transduction process of glucagon prior to cAMP production.

The GK rat is used widely as an animal model of genetically induced type-2 diabetes. Previous studies using the euglycaemic clamp have shown that hepatic glucose production is increased in this animal in vivo [2, 13], although the responsible mechanism has not yet been totally clarified. The present study showed directly that basal glucose output was enhanced in the perfused GK rat liver, i.e. under conditions excluding the effects of humoral factors (Fig. 1). This increase in basal glucose output does not seem to be mainly due to the glycogen content in the GK rat liver, since this was not significantly different from that in control (Table 1). Light-microscopic observation of hepatic glycogen stained using the periodic-acid-Schiff (PAS) reaction shows no difference between control and GK rats (Y. Doi, M. Iwai, B. Matsuura, M. Onji; unpublished data) and the rate of glycogenolysis may not be altered in the GK rat, since the activity of rate-limiting enzyme in glycogenolysis, glycogen phosphorylase, is also unchanged (preliminary experiments). However, as shown in the electron micrograph of Fig. 2, the distribution of glycogen particles in hepatocyte is almost homogenous in control, whereas glycogen particles seem to be located near plasma membrane in the GK rat hepatocyte. Although it is unknown whether the intracellular distribution of glycogen particle influences hepatic glucose output, the enhanced output of glucose from the perfused GK rat liver may be caused by several factors including heterogeneous distribution of glycogen within hepatocytes.

Metabolic zonation in the liver plays an important role in liver function [9]. A recent report has shown region-specific metabolism of glucose in periportal and perivenous hepatocytes isolated from BB rats, a model of insulin-dependent diabetes [6]. Although the metabolic zonation in the liver of GK rats has not yet been examined, it is possible that changes in hepatocytes heterogeneity during the development of diabetes may also be implicated in the enhancement of basal glucose output, an aspect that deserves future analysis.

In the perfused liver, glucagon dose-dependently increased glucose output, as shown in Fig. 1. This increase in glucose output seemed to be induced mainly through glycogenolysis, since the effect of glucagon was abolished after a 24-h fast (data not shown), which depletes liver glycogen [7]. However, a change in glycogen content in the perfused liver after glucagon infusion was not detectable, probably due to the small amount of glycogen degraded during the 5-min stimulation with glucagon (preliminary experiments). In the present study, the effect of glucagon on glucose production was not significantly different between control and GK rats (Fig. 1). Thus, the sensitivity of liver to glucagon was not altered in the diabetic GK rat.

Hyperglycaemia in diabetes is thought to be due mainly to reduced glucose-uptake in peripheral tissues, like skeletal muscle and adipose tissue, that express the insulin-responsive glucose transporter, GLUT4 [10, 11, 16]. Our results suggest that insulin resistance in the liver may also play an important role in the regulation of blood glucose level. In the perfused liver, glucagon-induced glucose output was inhibited significantly by insulin in control rats both at lower (0.06 nM) and higher (1.0 nM) glucagon concentrations (Fig. 3A and B). This antagonistic effect of insulin on glucagon-induced glucose output was absent in the liver of the GK rat, thus demonstrating insulin resistance in the liver (Fig. 3C and D). Given that the plasma glucagon concentration is not altered in adult GK rats [12] and that the effect of glucagon on glucose production appeared to be similar in both control and GK rats, since the increase in glucose output from basal level caused by glucagon was not different in both groups (Fig. 1), the loss of the antagonistic action of insulin on the effect of glucagon on hepatic glucose production may be involved in the hyperglycaemia in GK rats.

Although the number of insulin receptors in the GK rat liver is decreased to 20–30% [2], this does not seem to be the major mechanism of hepatic insulin resistance in this animal [2]. The kinase activity of insulin receptors is not changed in GK rats, suggesting that liver insulin resistance is mainly accounted for by a post-receptor defect [2]. In the present study, the glucagon-induced increase in cAMP production was significantly inhibited by insulin (Fig. 4) and this insulin action was attenuated clearly in the GK rat (Fig. 4). Although cAMP production in the pancreatic islets of GK rat is enhanced [1], the

basal level of cAMP in the perfused liver was not significantly different between control and GK rats (Fig. 4). The effect of glucagon on cAMP content was not altered in the GK rat, while insulin reduced the increase in cAMP content during glucagon infusion in control, but not GK rats (Fig. 4). These results suggest that the antagonistic action of insulin on glucagon-induced glucose release occurs at the level of signal transduction prior to cAMP production, an aspect which deserves further investigation.

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