ENHANCED PERIPHERAL GLUCOSE UTILIZATION AND SUPPRESSED HEPATIC GLYCOGEN SYNTHESIS IN KK MICE TREATED WITH ISLET-ACTIVATING PROTEIN (IAP)

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Effect of islet-activating protein (IAP) on glucose utilization of peripheral tissues and glycogen synthesis of the liver was investigated in diabetic KK mice. The ^{14}C -content of diaphragm glycogen following the injection of $[\text{U-}^{14}\text{C}]$ glucose was much less in KK mice than in dd/Y mice, and it was restored to normal in KK mice treated with IAP. The enhanced glucose utilization in these mice was completely abolished by the injection of anti-insulin serum. Glycogen synthesis of the liver after glucose load was also less in KK mice than in dd/Y mice, and it was clearly suppressed in both groups of mice by IAP treatment.

It was concluded that improved glucose tolerance of IAP-treated KK mice was mostly due to enhanced glucose utilization of peripheral tissues secondary to increased insulin secretion induced by IAP injection.

Keywords—islet-activating protein; peripheral glucose utilization; hepatic glycogen synthesis; glucose tolerance; ^{14}C -glucose; KK mice

INTRODUCTION

KK mice are genetically hyperglycemic, hyperinsulinemic and obese.^{1,2)} In a previous paper,³⁾ we showed that a single injection of islet-activating protein (IAP), a new protein purified from the culture medium of *Bordetella pertussis*,⁴⁾ into KK mice was very effective in eliminating their diabetic symptoms over a long period of time. This effect of IAP was partly, though not fully, accounted for by enhanced insulin secretion which was very similar to our previous observations with normal rats⁵⁻⁷⁾ and diabetic rats.⁸⁾

One of the diabetic symptoms characteristic of KK mice was impaired glucose tolerance. It was mostly normalized by IAP treatment.³⁾ While it is assumed that glucose tolerance pattern mainly reflects peripheral glucose utilization, the liver is also known to play an important role in disposal of orally as well as intravenously administered glucose.^{9,10)}

The aim of the present paper is thus to investigate the relative contribution of peripheral glucose utilization and hepatic glycogen synthesis

to glucose tolerance in IAP-treated KK mice. It was shown that IAP treatment resulted in enhancement of glucose utilization in peripheral tissues and in suppression of glucose-induced increase in glycogen synthesis in the liver. We concluded that improved glucose tolerance of IAP-treated KK mice was mostly due to enhanced peripheral glucose utilization secondary to increased insulin secretion induced by IAP injection.

MATERIALS AND METHODS

Five- to six-month-old male KK mice, weighing 35–40 g, were used throughout the present experiments; a litter of mice derived from Nagoya University was bred in our laboratory. In some experiments, male mice of the dd/Y strain were used as non-diabetic control.

IAP (5 $\mu\text{g}/\text{kg}$) or saline (as control) was injected intravenously into the tail vein of mice 5 to 10 d before experiments. During this period, glucose tolerance pattern was kept normal even in KK mice.³⁾ 5-Methoxyindole-2-carboxylic acid

(MICA) was injected intraperitoneally at a dose of 25 or 50 mg/kg into 20-h fasted mice. Immediately before and after the injection of MICA, blood samples were withdrawn from the infra-orbital sinus of mice and analyzed for glucose by the glucose oxidase method.¹¹⁾ Glucose utilization by peripheral tissues *in vivo* was investigated by measuring the incorporation of [U-¹⁴C]glucose into glycogen of the diaphragm muscle and glyceride-glycerol of the epididymal adipose tissues as that described by Stauffacher and Renold.¹²⁾ The interval between intraperitoneal injection of [U-¹⁴C]glucose (0.6 μ Ci/10 g) and sacrifice (for sampling tissues) was 30 min. At sacrifice, blood samples were also taken to be analyzed for insulin by the double antibody method¹³⁾ and for specific activity of glucose by the method described elsewhere.¹⁴⁾ To obtain the relative conversion of glucose to both metabolites, the ¹⁴C-content of glycogen or glyceride-glycerol was divided by specific activity of glucose in the blood at sacrifice.¹⁵⁾ In some experiments, anti-insulin serum was injected into the tail vein of mice to neutralize circulating insulin 20 min prior to the injection of [U-¹⁴C]glucose. In order to investigate glycogenic activity in the liver, [U-¹⁴C]glucose (0.4 μ Ci/10 g) with or without glucose (50 mg/10 g) was injected into the tail vein 30 min before killing. The ¹⁴C-content of glycogen was determined as described elsewhere.¹⁴⁾ The glycogenic activity was calculated by dividing the ¹⁴C-content of glycogen by the specific activity of blood glucose as described by Shikama *et al.*¹⁶⁾

IAP was purified from the 48-h culture medium of *Bordetella pertussis* according to the procedure described elsewhere.⁴⁾ Anti-insulin serum was obtained from guinea pigs immunized with bovine crystalline insulin.¹⁶⁾ Sources of other reagents are as follows; 5-methoxyindole-2-carboxylic acid (MICA); Aldrich Chemical Co., [U-¹⁴C]glucose; New England Nuclear; bovine crystalline insulin; Shigma Co. Reagents for radio-immunoassay of insulin were purchased from Dianabot Radioisotope.

Statistical evaluation was carried out using Stu-

dent's *t* test.

RESULTS

Development of Marked Hypoglycemia in IAP-treated KK Mice following the Injection of a Gluconeogenic Inhibitor

It is well-known that most of the blood glucose arise from hepatic gluconeogenesis in the fasted state. The inhibition of gluconeogenesis by the injection of MICA, a potent inhibitor of gluconeogenesis,¹⁴⁾ resulted in a rapid decline of the blood glucose in KK mice (Fig. 1), presumably as a result of uptake of blood glucose into peripheral tissues. Prior treatment of KK mice with IAP lowered the fasting blood glucose level; the

FIG. 1. *Effect of MICA on the Blood Glucose Concentration in KK Mice*

KK mice which had been injected with saline (○, △, □) or IAP (5 μ g/kg i.v. ●, ▲, ■) 5 d before were injected i.p. with following doses of MICA (per kg body wt); ○, ● 0 mg (saline), △, ▲ 25 mg, □, ■ 50 mg. The number of observations is three. * $p < 0.05$, ** $p < 0.01$, difference between IAP-treated KK mice and control KK mice is significant.

difference in the blood glucose level between IAP-treated and nontreated mice was kept to a similar extent after the injection of MICA. Thus, suppression of hyperglycemia caused by IAP treatment would not solely be accounted for by inhibition of hepatic gluconeogenesis; enhanced

peripheral glucose uptake would also be involved. *Enhanced Glucose Utilization in Peripheral Tissues in Mice after IAP Treatment*

There was no difference in body weight and epididymal adipose tissue weight between non-treated and IAP-treated groups of KK or dd/Y

TABLE I. *Weight of Whole Body and Epididymal Adipose Tissues and Concentration of Plasma Insulin and Blood Glucose in KK Mice and dd/Y Mice with or without IAP Treatment*

		Body wt (g)	Wet wt of adipose tissue (g)	Blood glucose (mg/100 ml)	Plasma insulin (μ U/ml)
dd/Y					
Control	(5)	41 $\pm$ 2	1.0 $\pm$ 0.2	77 $\pm$ 4	12 $\pm$ 4
IAP-treated	(4)	42 $\pm$ 2	1.5 $\pm$ 0.3	67 $\pm$ 5	37 $\pm$ 11
KK					
Control	(5)	39 $\pm$ 1	1.2 $\pm$ 0.1	106 $\pm$ 5 ^{a)}	94 $\pm$ 4 ^{b)}
IAP-treated	(7)	41 $\pm$ 1	1.1 $\pm$ 0.1	80 $\pm$ 3 ^{d)}	144 $\pm$ 20 ^{b), c)}

All mice which had been injected saline (control) or IAP (5 μ g/kg i.v.) 5 d before were sacrificed by decapitation. The number of observations is shown in parentheses. a) $p < 0.05$, b) $p < 0.01$; difference between dd/Y and KK mice is significant. c) $p < 0.05$, d) $p < 0.01$; effect of IAP treatment is significant.

TABLE II. *The Incorporation of 14 C from [U- 14 C]glucose into Diaphragm Glycogen and Epididymal Glyceride-glycerol*

Anti-insulin serum	Diaphragm glycogen (μ mol/g tissue)		Epididymal glyceride-glycerole (μ mol/g tissue)	
	—	+	—	+
dd/Y				
Control	60 $\pm$ 20 (8)	5 $\pm$ 1 (4) ^{e)}	30 $\pm$ 6 (8)	16 $\pm$ 3 (4)
IAP-treated	326 $\pm$ 98 (8) ^{c)}	10 $\pm$ 3 (3) ^{f)}	415 $\pm$ 87 (8) ^{d)}	14 $\pm$ 2 (3) ^{f)}
KK				
Control	13 $\pm$ 5 (9) ^{a)}	6 $\pm$ 1 (5)	31 $\pm$ 3 (9)	30 $\pm$ 6 (5)
IAP-treated	63 $\pm$ 20 (11) ^{a), c)}	11 $\pm$ 5 (5) ^{e)}	259 $\pm$ 74 (11) ^{d)}	24 $\pm$ 5 (5) ^{f)}

Mice of KK strain or dd/Y strain which had been injected with saline (control) or IAP (5 μ g/kg i.v.) 5 d before were first injected i.v. with anti-insulin serum or saline and then injected with [U- 14 C] glucose (0.6 μ Ci/10 g) i.p. 30 min later. At 30 min after the 14 C injection, all mice were sacrificed by decapitation and 14 C-contents of both metabolites were determined. The results are expressed as the conversion of 14 C-glucose into both metabolites after being corrected for by the specific activity of blood glucose as shown in MATERIALS and METHODS. The number of observations is shown in parentheses. a) $p < 0.05$, b) $p < 0.01$; difference between dd/Y mice and KK mice is significant. c) $p < 0.05$, d) $p < 0.01$; effect of IAP treatment is significant. e) $p < 0.05$, f) $p < 0.01$; effect of anti-insulin serum is significant.

mice (Table I). The injection of IAP caused a three fold increase in plasma insulin concentration in dd/Y mice and developed more hyperinsulinemia in KK mice. The blood concentration of glucose was again lower in IAP-treated KK mice than in control KK mice, but no difference was observed after IAP treatment of dd/Y mice.

As shown in Table II, the incorporation of ^{14}C -glucose into diaphragm glycogen of KK mice was much less than that of dd/Y mice before IAP treatment despite a comparable incorporation into epididymal glyceride-glycerol. Treatment with IAP caused a 5-fold increase in the ^{14}C -content of diaphragm glycogen in both KK and dd/Y mice. As a result, there was a great difference between these strains of mice even after IAP treatment. No significant difference was observed

in the ^{14}C -content of epididymal glyceride-glycerol between KK and dd/Y mice; IAP caused marked increases in the ^{14}C -contents in both groups.

The effect of IAP to increase the incorporation of ^{14}C into diaphragm glycogen and epididymal glyceride-glycerol was completely abolished by prior injection of anti-insulin serum to the mice. This strong action of anti-insulin serum was observed in either KK or dd/Y mice. After anti-serum treatment, there was no difference in ^{14}C -content of glycogen between KK and dd/Y mice regardless of whether the mice had been injected with IAP or not. Thus, enhancement of peripheral glucose utilization which is characteristic of IAP treatment would be mostly explained in terms of hyperinsulinemia.

TABLE III. *Effect of IAP Treatment on Specific Activity of Glucose in Blood, and Glycogen Content, Specific Activity of Glycogen and Glycogenic Activity in the Liver*

	Blood		Liver	
	Specific activity of glucose (dpm $\times 10^{-3}$ /mg)	Glycogen content (mg/g tissue)	Specific activity of glycogen (dpm $\times 10^{-3}$ /mg glycogen)	Glycogenic activity ($\mu\text{mol/g tissue}$)
Exp. 1 (without glucose)				
dd/Y				
Control	(4) 41.0 ± 12	0.29 ± 0.10	17.3 ± 1.8	1.35 ± 0.8
IAP-treated	(4) 33.8 ± 6.6	0.40 ± 0.10	11.5 ± 1.2^c	1.40 ± 0.4
KK				
Control	(5) 35.1 ± 4.1	0.35 ± 0.06	12.8 ± 1.3	1.48 ± 0.6
IAP-treated	(5) 30.3 ± 7.4	0.29 ± 0.06	9.8 ± 1.5	0.65 ± 0.2
Exp. 2 (with 50% glucose)				
dd/Y				
Control	(4) 28.7 ± 5.4	6.4 ± 1.0	22.0 ± 3.3	32 ± 6
IAP-treated	(4) 23.1 ± 7.7	0.6 ± 0.1^d	4.8 ± 1.4^d	0.9 ± 0.3^d
KK				
Control	(4) 19.3 ± 3.7	2.2 ± 0.5^b	24.8 ± 5.7	11 ± 4^a
IAP-treated	(4) 20.8 ± 3.9	0.6 ± 0.1^c	7.4 ± 0.8^c	1.3 ± 0.4^c

Fasted KK mice or dd/Y mice which had been injected with saline (control) or IAP ($5 \mu\text{g/kg i.v.}$) 5 d before were injected with [^{14}C]-glucose ($0.4 \mu\text{Ci/10 g}$) combined with saline (Exp. 1) or 50% glucose (Exp. 2, 0.1 ml/10 g) and sacrificed by decapitation 30 min later. The number of observations is shown in parentheses. a) $p < 0.05$, b) $p < 0.01$; difference between dd/Y mice and KK mice is significant. c) $p < 0.05$, d) $p < 0.01$; effect of IAP treatment is significant.

Suppression of Glycogen Synthesis in the Liver of IAP-treated Mice

The injection of IAP into dd/Y mice or KK mice caused an increase in the fasting concentration of plasma insulin. Since insulin is known to directly stimulate glycogen synthesis in the liver,¹⁷⁾ it is likely that improved glucose tolerance of IAP-treated mice is partly dependent on enhanced incorporation of glucose into hepatic glycogen by the action of increased insulin. We, therefore, studied the effect of IAP on glycogen synthesis in the liver of fasted or glucose-loaded mice (Table III). If glucose was not administered to mice, no significant differences were observed in glycogen contents and glycogenic activities between all groups, except for a slight decrease in specific activities of glycogen induced by IAP in dd/Y mice (Table III-Exp. 1). The effect of diabetes and IAP became evident when mice was loaded with 50% glucose (Table III-Exp. 2). There were no significant differences in specific activities of blood glucose as a precursor of hepatic glycogen among any groups of mice. KK mice showed less glycogenic activity and glycogen content than dd/Y mice before IAP treatment. IAP treatment produced a marked decrease in glycogen contents, specific activities and glycogenic activities in KK mice as well as in dd/Y mice. There were no significant differences between the two strains in any of these parameters. These findings suggest that under normal conditions a considerable fraction of *i.v.* injected glucose serves as a substrate of hepatic glycogenesis, and that essentially no glucose was converted into liver glycogen after IAP treatment either in dd/Y mice or KK mice.

DISCUSSION

The ¹⁴C-content of diaphragm glycogen following the intraperitoneal injection of ¹⁴C-glucose was much less in KK mice than in dd/Y mice, and IAP treatment was very effective in restoring to normal ¹⁴C-content in KK mice. On the other hand, there were no significant differences in the ¹⁴C-contents of epididymal glyceride-glycerol between KK mice and dd/Y mice

regardless of whether the mice had been injected with IAP or not. Such changes in the ¹⁴C-content of the muscle were in agreement with our previous data³⁾ that glucose tolerance of KK mice was markedly inferior to that of dd/Y mice, and the treatment of KK mice with IAP was very effective in normalizing their impaired glucose tolerance. The injection of anti-insulin serum, which is well-known to produce acute diabetes in experimental animals, caused a complete abolition of the incorporation of ¹⁴C-glucose into diaphragm glycogen. In view of these findings, glucose tolerance is partly though not fully, dependent on glucose utilization of the muscle, and the curative effect of IAP to diabetic animals could be explained by the enhanced utilization. These ideas are also supported by the finding that MICA-induced hypoglycemia still appeared in IAP-treated KK mice even when MICA attenuated glucose release through inhibition of gluconeogenesis. The enhanced peripheral utilization of glucose could be mostly explained by hyperinsulinemia induced by IAP since the injection of anti-insulin serum abolished the effect of IAP treatment. The reason why glucose tolerance correlates to glucose utilization of the muscle rather than that of adipose tissues remained unclear.

The administration of glucose caused an increase in hepatic glycogen content in nontreated dd/Y mice and the effect of diabetes on glycogen synthesis became first evident. Glycogen content and glycogenic activity of the liver were much less in KK mice than in dd/Y mice. Thus, it is clear that part of glucose loaded accelerated hepatic glycogen synthesis. These findings are in agreement with the reports^{18,19)} that glucose administration caused a rapid increase in hepatic glycogen synthesis and it was ineffective in diabetic animals. Shikama and Ui²⁰⁾ suggested that the precursors of hepatic glycogen after glucose loading were not always the loaded glucose itself, but the increased content of glycogen was partly due to that a final product of gluconeogenesis was converted to hepatic glycogen from blood glucose by the hyperglycemia induced by glucose loading. In any case, glucose

tolerance test would be also influenced partly by the ability of the liver to synthesize glycogen as one of ways in disposal of administered glucose.

The treatment of both groups of mice with IAP resulted in a marked suppression of glucose-induced activation of glycogen synthesis. These findings apparently seemed to be contrary to improved glucose tolerance shown in IAP-treated mice.³⁾ However, similar results were reported in pertussis-sensitized rats²¹⁾ and it was further demonstrated that hyperinsulinemia of these rats was responsible for suppression of hepatic glycogen synthesis. IAP is the factor responsible for the hyperinsulinemic action of pertussis vaccine.⁴⁻⁶⁾ Although insulin is known to activate glycogen synthesis¹⁷⁾ and to inhibit gluconeogenesis²²⁾ in the liver directly, it is also true that the injection of insulin into normal animals²¹⁾ or man²³⁾ causes inhibition of glycogen synthesis and promotion of glucose production explained by the secondary action of hypoglycemia rather than by the direct action of insulin. Conceivably, the liver of IAP-treated mice, like that of insulin injected, would exclusively release glucose through gluconeogenesis into the blood at the expense of glycogenesis to antagonize the enhanced peripheral glucose utilization due to the hyperinsulinemia.

Acceleration of hepatic glycogen synthesis induced by glucose injection in non IAP-treated mice would be due to an increased substrate availability and a glycogenic action of insulin moderately increased. On the other hand, glucose was known to cause more marked hyperinsulinemia in IAP-treated mice than in nontreated mice, suggesting extraordinary enhancement of glucose utilization in peripheral tissues of IAP-treated mice. Under these conditions, most of glucose injected would be consumed in peripheral tissues and it would not accelerate glycogen synthesis in the liver. Thus, glucose injection revealed marked difference in glycogen metabolism of the livers between nontreated and IAP-treated mice. In this respect there was no difference between dd/Y and KK mice.

In conclusion, enhanced peripheral glucose

utilization was primarily responsible for improved glucose tolerance in IAP-treated KK mice. Under such a hyperinsulinemic condition, glycogen synthesis in the liver would not play an important role in disposal of administered glucose.

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