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Effects of Phlorizin and Acipimox on Insulin Resistance in STZ-Diabetic Rats

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To evaluate the roles of hyperglycemia and increased plasma FFA level in the development of insulin resistance, we examined the effects of phlorizin and acipimox treatments on tissue sensitivity to insulin in streptozotocin(STZ)-diabetic rats.

Insulin sensitivity was assessed with the glucose-insulin clamp technique. Blood glucose concentration was clamped at basal levels of control and diabetic states, and plasma insulin concentrations were clamped at the levels of basal, ~60 and ~1500 μU/ml.

In diabetic rats, the basal blood glucose and plasma FFA levels in the fasting state were elevated, while the plasma insulin concentration was lower than in normal controls. Moreover, diabetic rats became glucose intolerant after intravenous injection of glucose. The metabolic clearance rate(MCR) of glucose showed a decrease of basal and insulin stimulated response in diabetic rats. As results of the glucose-insulin clamp study and intravenous glucose tolerance test, insulin resistance was developed in STZ-diabetic rats. Phlorizin treatment of diabetic rats recovered insulin sensitivity to nearly normal levels and improved glucose tolerance, but had no effect on insulin action in controls. Insulin sensitivity was also improved by acipimox treatment in diabetic rats, but did not reach normal levels.

These results show that hyperglycemia is an obvious causative factor of insulin resistance, and increased FFA level may also act on the development of insulin resistance in STZ-diabetic rats.

Key Words : *Hyperglycemia, Increased FFA level, Glucose-insulin clamp, STZ-diabetic rats, Phlorizin, Acipimox.*

INTRODUCTION

Insulin resistance has been demonstrated as a prominent feature in both human and animal models of IDDM and NIDDM(Karnieli et al., 1981 ; DeFronzo et al., 1982 ; Beck-Nielsen et al., 1984 ; Blondel et al., 1989). However, the pathogenetic sequence of events leading to the emergence of the defect in insulin action remains controversial. In the most general sense, the insulin resistance could be either primary or acquired.

If insulin resistance is developed by secondary causes, associated metabolic derangements in the diabetes have been postulated as possible causes. Among the metabolic derangements of the diabetic

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state, much attention is paid to hyperglycemia and increased plasma FFA as the causative factors that have an effect on peripheral glucose utilization and insulin sensitivity.

Hyperglycemia has been postulated by some authors to be the most important aggravating factor involved in the impairment of insulin action in NIDDM(Unger and Grundy, 1985). This hypothesis is supported by the experimental data of Rossetti et al.(1987) and Blondel et al.(1990) suggesting that hyperglycemia can lead to the development of insulin resistance. However, Laury et al.(1989) demonstrated that short-term(4 days) hyperglycemia and hyperinsulinemia do increase tissue sensitivity to insulin.

Some other results have introduced the role of fat metabolism. According to Lee et al.(1988) and Randle et al.(1988), it is clear that a rise in plasma FFA causes the insulin resistance.

In the present study, we examined the effect of hyperglycemia and increased concentration of plasma FFA on insulin resistance in STZ-diabetic rats.

MATERIALS AND METHODS

Experimental protocol

Sprague-Dawley female rats, 6 week of age and weighing ~2000g, were used. These rats were divided into six groups : normal control, normal treated with phlorizin, normal treated with acipimox, STZ treated diabetes, diabetes treated with phlorizin(which normalizes blood glucose without change in insulin level), and diabetes treated with acipimox-(which has a lowering effect on plasma FFA level), were studied.

Diabetes was induced by intraperitoneal injection of STZ(50 mg/kg BW, Sigma) in citrate buffer (pH 4.5). We used diabetic rats with glucosuria on the second day of STZ injection.

Phlorizin(0.4 g/kg body weight per day ; made up as a 20% solution in propylene glycol, Sigma) treatment was initiated on the second day after STZ injection in urine sugar positive rats and was continued for 8 days. Phlorizin, which inhibits renal tubular glucose reabsorption, was subcutaneously administered b.i.d..

Acipimox(0.1 g/kg body wt per day ; suspended in normal saline, Olbetam® Farmitalia, Italy) treatment was given using the same method as phlorizin, but the route of administration was per oral. Acipimox decreases the level of plasma FFA by inhibiting hormone sensitive tissue lipase.

Glucose-Insulin clamp study

At 8 a.m., after an overnight fast, the rats were anesthetized with intraperitoneal injections of pentothal sodium(40 mg/Kg BW). Tracheotomy was done to prevent respiratory difficulty.

Two catheters were inserted into a femoral vein to infuse insulin and [3-^{3}H]glucose(New England Nuclear) using an infusion pump(Harvard Apparatus), and another catheter was also inserted into the other femoral vein to infuse 20% glucose solution through a peristaltic pump(Minipuls 3, Gilson). One more catheter was inserted into the left femoral artery to take a blood sample for measuring blood glucose, insulin, and specific activity of tritiated glucose.

At time zero, a prime(4 μCi)-continuous(0.2 μCi/min) infusion of [3-^{3}H]glucose was initiated and continued throughout the study(about 180 min). At this time, blood glucose and plasma insulin level were maintained at fasting basal levels. Plasma samples for the determination of tritiated glucose specific activity and insulin were obtained at 5 min intervals from 50 to 60 min of adjusted insulin levels. Then a continuous(1.7mU/kg.min) infusion of regular insulin (Velosulin, Nordisk) was given for 1 hr between 60 and 120 min to maintain the plasma insulin concentration at ~60 μU/ml. At 120 min, the continuous insulin infusion rate was increased to 34 mU/kg.min to maintain the plasma insulin concentration at ~1500 μU/ml. The insulin was dissolved in normal saline with 1% bovine serum albumin. In all groups a variable infusion of 20% glucose was started at time 60min. The level of glucose was clamped at the fasting basal level of individual rats through glucose infusion adjusted by negative feedback mechanism using a peristaltic pump. Blood sampling for glucose determination was done at 5 min intervals from 0 to 180 min. The blood glucose concentration was determined immediately by glucometer(Glucoscot, DIC). The clamped levels of glucose were about 100 mg/dl in normal and phlorizin treated diabetic rats, and were about 250 to 450 mg/dl in diabetic control and acipimox treated diabetic rats.

To define the effect of glycemic level on metabolic clearance rate, we elevated blood glucose level to 300 mg/dl within 30-45 min in the normal rats,

and then a hyperglycemic hyperinsulinemic clamp was performed for an additional 120 min the same as in the other experimental groups.

To prevent intravascular volume depletion, we reinfused packed RBC dissolved in normal saline after collection of plasma. At the end of the three-step insulin clamp study, urine was collected and measured for tritiated glucose. The measurement of tritiated glucose was done with a liquid scintillation counter(LKB, Sweden).

Calculation of metabolic clearance rate(MCR) and hepatic glucose production rate(HGP)

Since the clamped levels of glucose were different among groups, we used MCR as an index of insulin sensitivity. MCR means the value of glucose disappearance rate(Rd) divided by plasma glucose concentration(Andrews et al, 1984). Rd(mg/min) was calculated by the method below (Kergoat, 1985).

$$Rd = \frac{[3\text{-}^{3}H]\text{glucose infusion rate(CPM/min)}}{\text{steady-state value of glucose specific activity(CPM/mg)}}$$

The steady-state value of glucose specific activity was the ratio of radioactivity in plasma to plasma glucose concentration. Since we determined whole blood glucose concentration, the plasma glucose concentration was calculated by the equation below(Tobin et al, 1993).

$$\text{Plasma glucose} = \text{whole blood glucose}/(1-[2.4\times10^{-3}\times HCT\%]).$$

HGP was also calculated by the formula below.

$$HGP = Rd - \text{Exogenous glucose infusion rate}$$

Intravenous glucose tolerance test

The intravenous glucose tolerance test was performed under the pentothal anesthesia after overnight fasting. Blood samples for blood glucose and plasma insulin determination were obtained from the indwelling arterial catheter at 0, 5, 30, and 60 min. after 0.5 g/kg body weight glucose injection via the jugular vein.

Analysis

Insulin concentration was determined by radioimmunoassay. Concentrations of FFA, cholesterol and triglyceride were analyzed by diagnostic kits.

Statistical analysis

All values are presented as the mean S.E.. Student's t test was performed for statistical analysis. P values <0.05 were considered to be statistically significant.

RESULTS

In STZ diabetic rats, the blood glucose and plasma FFA levels in the fasting state were elevated(366 ±44.3 vs 96±9.4 mg/dl, 1017±88 vs 651±33 μ Eq/l, respectively), while the plasma insulin concentration was lower than in the normal rats(11.6±1.5 vs 21.5±3.9 μU/ml). But the level of blood glucose was normalized with treatment of phlorizin(104 ±3.5 mg/dl), and the level of plasma FFA was decreased by treatment with acipimox(251±29 μ Eq/l)(Table 1).

The concentration of triglyceride in the acipimox treated groups was lower than that of the control

Table 1. Baseline data of experimental groups

Group	n	wt gm	FBS mg/dl	FPI μU/ml	FFA μEq/l	TG mg/dl	Cholesterol mg/dl
NORMAL							
Control	7	210±9	96±9	21.5±3.9	651±33	29.7±4.2	37.4±3.9
Phlorizin	5	211±7	94±5	19.0±3.9	507±18*	33.2±5.0	45.6±6.8
Acipimox	6	204±9	105±6	16.6±3.2	245±26*	22.6±1.7*	35.8±3.2
DLABETIC							
Control	7	190±5	366±44*	11.6±1.5	1017±88*	30.9±2.1	34.8±4.6
Phlorizin	7	209±1	104±4	9.2±1.9*	706±100#	47.9±5.9*,#	38.9±2.8
Acipimox	7	194±6	358±38*	8.0±1.2*	251±29*,#	22.6±1.2*,#	29.9±2.2#

All values are mean±S.E.. n, number of cases ; wt, weight ; FBS, fasting blood glucose ; FPI, fasting plasma insulin ; FFA, free fatty acid ; TG, triglyceride. *P<0.05, vs normal control ; #P<0.05, vs diabetic control.

groups(22.6±1.2 vs 30.9±2.1 mg/dl), but the level of cholesterol was not significantly different according to experimental group.

During the IVGTT the incremental area under the curve and the rise in blood glucose concentration for 1 hr was ~60% as large in the normal controls compared with those in the diabetic controls, whereas the postglucose plasma insulin response was markedly impaired in the diabetic controls. In the phlorizin treated diabetic rats the fasting plasma glucose concentration was normalized, but the incremental area under the glucose curve was significantly higher than that in the normal controls(Fig. 1).

The postglucose plasma insulin response was absent in the diabetics, but there was an increasing tendency of insulin concentration at 5-min after glucose load compared with that of basal level in the phlorizin treated diabetics in spite of having no significant difference(18±3.6 vs 12±1.5 μU/ml).

The results of the three step insulin-clamp study are shown in Fig. 2 and Table 2. Steady state plasma insulin concentrations during both hyperinsulinemic steps were similar in all experimental groups. In diabetic controls, the dose response curve of MCR of glucose revealed rightward and downward shifting compared with normal controls. This result indicated the development of insulin resistance.

Phlorizin treatment of diabetic rats recovered insulin sensitivity to nearly normal level. But, phlorizin treated diabetics revealed mild glucose intolerance after IVGTT. Phlorizin itself had no effect on MCR of glucose in the normal rats.

Insulin sensitivity was also improved with acipimox treatment, but failed to reach normal levels in the diabetic rats. The glucose tolerance curve of the acipimox treated diabetics was not changed compared to the diabetic controls.

DISCUSSION

Rats were made diabetic by intraperitoneal injection of STZ, which resulted in moderate fasting

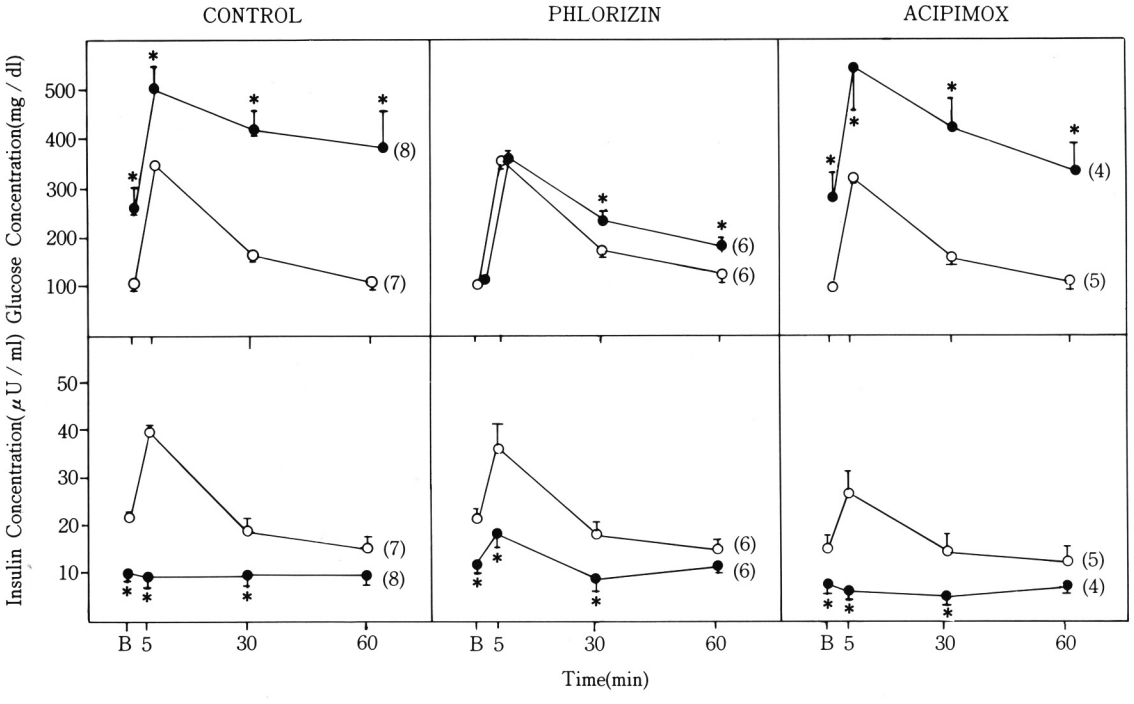

Fig. 1. IVGTT in normal(○) and diabetic(●) rats. 0.5g/kg body weight of glucose was given intravenously immediately after blood sampling for overnight fasting basal values(B). Each value is mean±S.E.. The number in parentheses indicates experimental cases. * $P<0.05$, vs control.

Table 2. Steady state insulin(SSPI) and glucose concentrations(SSPG), glucose infusion rate(SSGI), and hepatic glucose production(HGP) during the glucose insulin clamp studies in normal and diabetic rats

Group	n	Insulin Infusion rate mU/kg·min	SSPI μU/ml	SSPG mg/dl	SSGI mg/kg·min	HGP mg/kg·min
MORMAL						
Control	7	0	21.5±3.9	103±6		10.01±0.40
		1.7	59.0±3.2	99±4	7.35±0.90	6.15±0.65
		34	1456.0±52.8	101±4	18.09±1.00	1.95±1.90
Phlorizin	5	0	19.0±3.9	191±3		7.45±0.40*
		1.7	54.2±2.6	98±5	5.20±0.75	4.90±0.75
		34	1608.1±51.2	102±4	17.90±2.70	1.40±1.50
Acipimox	6	0	16.6±3.2	91±6		8.60±0.20*
		1.7	58.8±2.9	101±4	8.85±0.50	5.30±0.80
		34	1428.3±53.3	101±5	19.25±1.15	4.05±1.50
DIABETIC						
Control	7	0	11.6±1.5	409±54		17.45±1.70*
		1.7	58.9±2.5	389±43	6.30±1.25	10.15±1.75*
		34	1472.3±73.8	374±45	19.60±1.65	5.75±1.85
Phlorizin	7	0	9.2±1.9	101±4		8.70±0.65
		1.7	61.7±4.1	104±4	6.10±0.75	5.80±0.70
		34	1496.0±64.0	104±4	15.35±0.50#	4.20±1.45
Acipimox	7	0	8.0±1.2	297±27		19.00±2.35*
		1.7	60.6±3.0	336±30	13.70±1.65*,#	8.60±1.35#
		34	1593.3±63.7	337±25	28.40±2.60*,#	1.85±1.00

All values are mean±S.E.. n indicates number of cases; *P<0.05, vs normal control; #P<0.05, vs diabetic control.

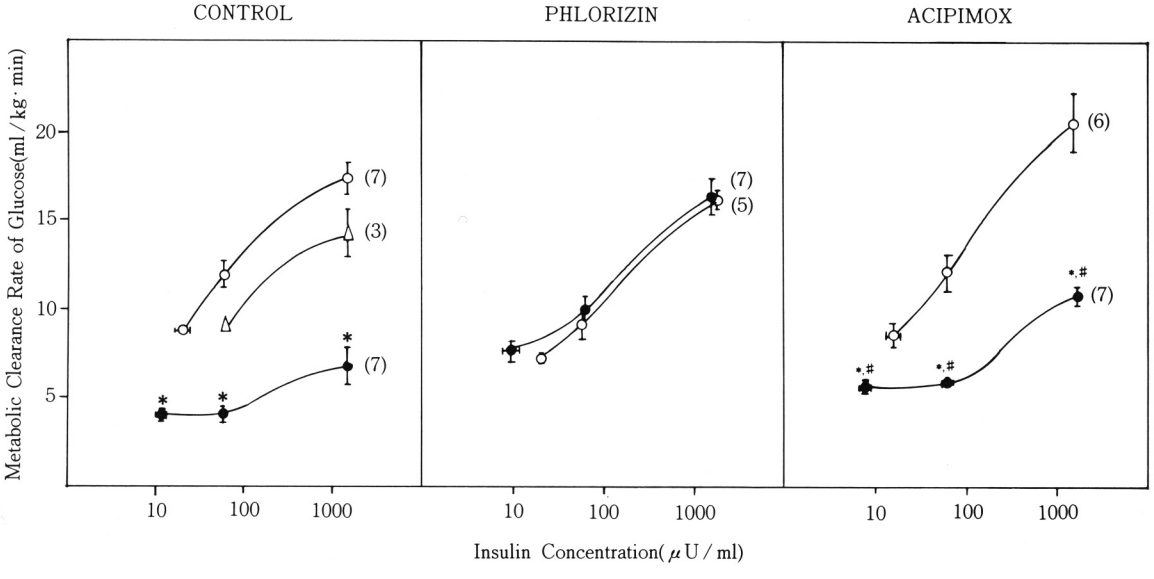

Fig. 2. Effect of phlorizin or acipimox on metabolic clearance rate of glucose in glucose-insulin clamped normal(○) and diabetic (●) rats. To define the effect of glycemic level on MCR, we compared MCR of experimental groups with that of the hyperglycemic(300mg/dl) clamped normal(△) rats that was induced by glucose infusion during 30-40 minutes. Each value is mean±S.E.. The number in parentheses indicates experimental cases. *P<0.05, vs control; #P<0.05, vs diabetic control.

hyperglycemia and glucose intolerance after IVGTT. Fasting insulin levels and response to glucose load were decreased in diabetic rats compared with those in normal rats. Tissue sensitivity to insulin was reduced by 40 to 50%. These results indicated that insulin resistance was developed in STZ-diabetic rats by initial defect in insulin secretion, and this was consistent with previous observations(Levy et al, 1984 ; Kergoat and Portha, 1985 ; Blondel et al, 1989). According to DeFronzo et al.(1985, 1981), the cause of insulin resistance in diabetic state seems to be a defect in the muscle tissue, because, under hyperinsulinemic conditions, the majority of infused glucose is used by muscle tissues.

In spite of the fact that the clamped glucose level in the diabetic control was three times greater than in the normal control, the glucose infusion rate during hyperinsulinemic clamp was the same in the normals(7.35±0.90 and 18.09±1.00 mg/kg • min) and in the diabetic controls(6.30±1.25 and 19.60± 1.65 mg/kg • min). This result means that fasting glucose level was set and maintained correlating to net disposal of glucose to tissue.

To define the effect of hyperglycemia on the development of insulin resistance, rats were injected with phlorizin daily to induce a state of persistent renal glucosuria. Fasting plasma glucose concentration was normalized in phlorizin treated diabetics, but the incremental area under the glucose curve in IVGTT was slightly greater in the phlorizin treated diabetics compared with the phlorizin treated normals. According to the results of the glucose-insulin clamp study, insulin sensitivity was recovered to nearly normal level in phlorizin treated diabetics. And, insulin sensitivity and glucose tolerance in the phlorizin treated normal rats were not significantly different to those in the normal controls. Thus, the cause of the improvement in insulin action in diabetic rats after phlorizin administration is not the effect of phlorizin itself but the effect of glycemic control, and this improvement in insulin action is resulted from an enhancement in tissue glucose uptake and not from a more effective suppression of hepatic glucose output.

These results suggest that hyperglycemia exerts a deleterious effect on insulin mediated glucose disposal in vivo. The cause of the mild glucose intolerance despite recovered insulin sensitivity in the phlorizin treated diabetics may be related to an impaired secretory response of insulin to hyperglycemia.

To define the effect of increased plasma FFA on the development of insulin resistance, we used acipimox which acts on adipocyte to inhibit lipolysis. Fasting plasma FFA concentration of the acipimox treated groups was decreased to ~40% of that of the normal control. In the acipimox treated diabetics, the tissue sensitivity to insulin was improved partially compared to that in the diabetic controls despite no significant difference between acipimox treated normals and normal controls, but glucose intolerance was not improved and the secretory response of insulin to hyperglycemia was absent. These results, together with the report(Lee et al, 1988 ; Randle et al, 1988) that said that a rise in plasma FFA causes insulin resistance, suggest that concentration of FFA correlates to the development of insulin resistance. According to several reports(Gomez et al, 1972 ; Fraze et al, 1985 ; Golay et al, 1987), increased FFA concentrations are related to defects in both the oxidative and nonoxidative pathways of glucose metabolism.

In acipimox treated groups, the plasma insulin concentration of fasting and postglucose loading was revealed to have a tendency to decrease compared to that in normal rats. But there was no statistically significant difference. It is necessary to confirm the effect of acipimox on insulin secretion.

These observations show that hyperglycemia is an obvious causative factor of insulin resistance, and increased FFA level may also act on the development of insulin resistance in STZ-diabetic rats.

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