Reduced Dihydroxyacetone Sensitivity and Normal Sensitivity to Glyceraldehyde and Oxidizing Agent of ATP-sensitive K^+ Channels of Pancreatic Beta Cells in NIDDM Rats

The inhibition of ATP-sensitive K^+(K_{ATP}) channels in pancreatic beta cells is a key step of insulin secretion induced by glucose. Glucose-induced insulin secretion from the beta cells is selectively impaired in patients with non-insulin-dependent diabetes mellitus(NIDDM) and in animal models of it. In order to clarify the site of this abnormal glucose response, we studied the effects of insulin secretagogues and sulfhydryl oxidizing agent, 2,2′-dithio-bis (5-nitropyridine) (DTBNP), on K_{ATP} channels in single beta cells of neonatally streptozotocin-induced NIDDM rats. We used the patch-clamp technique in cell-attached mode ($V_{pipette}$ = 0 mV). The inhibitory response to glucose of K_{ATP} channels was lacking in NIDDM rats, indicating reduced sensitivity to glucose of the channels. Glyceraldehyde (2~5 mM) in the diabetic beta cells elicited the same K_{ATP} channel inhibition as that obtained in controls. In contrast, dihydroxyacetone (DHA, 2~10 mM) sensitivity of K_{ATP} channels was significantly reduced in the beta cells of NIDDM rats. K_{ATP} channels in the diabetic beta cells were rapidly inhibited by 50 μM DTBNP, just as in the normal beta cells, suggesting that K_{ATP} channel function was normal. This indicates that one of the sites responsible for impaired glucose-induced insulin secretion in the pancreatic beta cells of NIDDM rats is located in the glycerol phosphate shuttle.
(JKMS 1997;12:286~92)

Key Words : *ATP-sensitive K^+ channel, Pancreatic beta cell, Glyceraldehyde, Dihydroxyacetone, Oxidizing agent, NIDDM rat, Patch-clamp technique*

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Received : March 21, 1997
Accepted : May 16, 1997

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* This work was supported by the Public Benefits Affairs Office of Korea Tobacco and Ginseng Corporation Research Fund.

INTRODUCTION

The ATP-sensitive potassium (K_{ATP}) channel of pancreatic beta cells plays an essential role in glucose-induced insulin secretion because it provides the link between glucose metabolism and membrane events. Inhibition of K_{ATP} channels and resultant depolarization are involved in the mechanisms leading to insulin secretion (1, 2). The channel is also inhibited by the hypoglycemic sulfonylureas (3), a class of drugs used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). This latter observation suggests that defective regulation of K_{ATP} channel may be one of causes of NIDDM.

It is well known that glucose-induced insulin secretion from the pancreatic beta cells is selectively impaired in patients with NIDDM (4) and in animal models of NIDDM (5). There are several reports that the reduced sensitivity to glucose in NIDDM is due to a defect of the glucose metabolism in the beta cells (6). However, the site of disturbed glucose metabolism is still open to question.

Neonatally streptozotocin-induced diabetic rats are commonly used as an NIDDM model (7, 8). In the present study, using the patch-clamp technique, we demonstrate that the site responsible for impaired glucose metabolism in the pancreatic beta cells of NIDDM rats is located in the glycerol phosphate shuttle, not in the pathway after glyceraldehyde-3-phosphate. In addition, we suggest that K_{ATP} channels in the beta cells of NIDDM are almost completely inhibited by the sulfhydryl oxidizing agents, as in the normal beta cells, indicating that K_{ATP} channel function is normal.

MATERIALS AND METHODS

Male Sprague-Dawley neonatal rats, 1.5 days old, received a subcutaneous injection of 70~90 mg/kg strepto-

zotocin (STZ) in 0.05 mol/L citrate buffer with pH 4.3 (NIDDM rats) or of an equivalent volume of citrate buffer alone (control rats). NIDDM and control rats were provided for experiments and cultures of islet cells between 8~12 weeks of age. Portha et al. (7) and recently, Tsuji et al. (5) have showed that this neonatally STZ-induced rat model exhibits features characteristic of NIDDM.

Measurements of blood glucose and serum insulin

Blood samples were collected via the tail capillary and the abdominal aorta for measurements of glucose and insulin, respectively. The capillary blood glucose level was determined by the glucose oxidase method (Medisense2, Medisense Inc., Waltham, MA, USA). The serum insulin level was measured by radioimmunoassay using the polyethylene glycol method (Amersham Co., Little Chalfont, Buckinghamshire, UK).

Preparation of cells

Islets of Langerhans were isolated by a collagenase digestion technique from the pancreas. Briefly, under ether anesthesia, we injected 3 ml neutral red solution (1 in 1000 w/v in 0.9 % saline) through the abdominal aorta after cutting inferior vena cava, since this stain is selective for islet tissue (9) and assists hand picking. Collagenase (1.2 mg/ml Hank's balanced salts solution, Sigma Chemical Co., St. Louis, MO, USA) solution was perfused through the pancreatic ducts retrogradely from the common bile duct. The separated pancreas was incubated in 37℃ water bath for 40 min. Dispersion of islet cells was accomplished according to the modified method of Gray et al. (9). Dispersed single cells were then transferred into RPMI 1640 medium (glucose concentration 11.1 mM, Sigma Chemical Co.) supplemented with fetal calf serum (10%), penicillin (100 u/ml), and streptomycin (0.1 mg/ml). They were cultured on small cover glasses (10 mm×3 mm) at 37℃ in a humidified incubator gassed with air and 5% CO_2. Individual cover glasses were transferred to the bath chamber on an inverted microscope (Carl Zeiss Corp., Jena, Germany) for patch-clamp experiments.

Electrophysiological study

We used the cell-attached mode of the patch clamp technique. Pipette was pulled from borosilicate glass and coated with Sylgard resin (Dow Corning, Midland, MI, USA) near the tip, fire-polished and had resistance between 3 and 5 MΩ. Single channel current was recorded at 0 mV pipette potential (V_p), using an Axopatch 200A patch-clamp amplifier (Axon Instruments Inc., Foster, CA, USA). During experiments the current and voltage signals were stored on video tape via a VR-10B pulse code modulator (Instrutech Corp., Great Neck, NY, USA) and later analyzed by pClamp 6.03 software (Axon Instruments Inc.). The channel activity was expressed as the mean patch current ($I=N\times P_o\times i$; Tsuura et al., 1992) (6) where N, P_o and i represent the number of channels in the patch membrane, the open probability of the channel, and the unit amplitude of the single channel current, respectively. The relative channel activity in the presence of testing materials was expressed as I/I_c, where I_c is the mean patch current recorded in control solution.

In the cell-attached experiments, the cells were bathed in a solution composed of (in mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, and 5 HEPES (pH 7.4 with NaOH). The pipette solution consisted of (in mM) 140 KCl, 2 $CaCl_2$, 10 HEPES, and 22.5 aspartate (pH 7.3 with KOH, 280 mOsm). The larger (>10 μM in diameter) of the dispersed cells were used for patch-clamp experiment because it is known that non-insulin-containing islet cells are characterized by their smaller size in rats (10). Electrophysiological experiments were performed at room temperature (22~25℃). Results were expressed as mean±SE (standard error). Statistical significance was evaluated by unpaired Student's t-test. All chemicals were from Sigma.

RESULTS

Blood glucose responses to subcutaneous glucose loading and serum insulin levels

As summarized in Table 1 and Fig. 1, fasting blood glucose levels in the control and NIDDM rats were 81.8 ±6.64 and 81.4±7.35 (mg/dl), respectively. After glucose loading, blood glucose levels in NIDDM group were significantly higher than those in the control group till 2 hours. Tsuji et al. (5) obtained similar result to ours with oral glucose loading. Serum insulin levels were not significantly different between the two groups.

Table 1. Fasting blood glucose and serum insulin levels in control and NIDDM rats

	Control rats (n=10)	NIDDM rats (n=9)
Fasting blood glucose (mg/dl)	81.8 ± 6.64	84.1 ± 7.35
Serum insulin (ng/ml)	0.75 ± 0.412	1.16 ± 0.181

Note : Data represent mean±SE. No difference between control and NIDDM rats was observed.

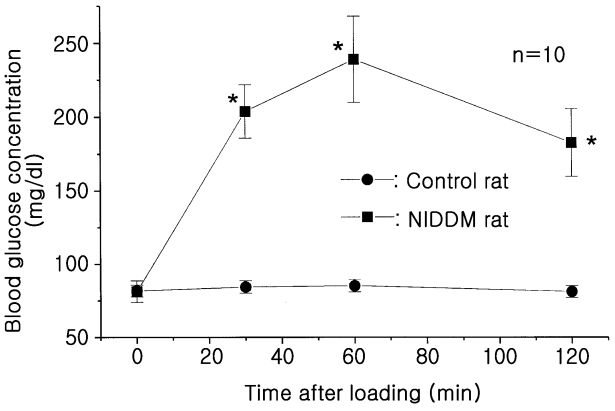

Fig. 1. Blood glucose responses to subcutaneous glucose loading (2 g/Kg) in control and NIDDM rats. * $p<0.05$ compared to values of control in each time. Mean±SE.

Body weight gain and daily water intake

Fig. 2 shows significant difference in body weight gain (A) which was observed between the two groups, particularly after 8 weeks of age. Daily water intake (B) was also larger in the NIDDM rats. Around 7 weeks of age, transient recovery of weight gain was observed. So we carried out the electrophysiological experiments between 8~12 weeks.

Electrophysiological results

The typical inhibitory effects of glucose on K_{ATP} channel activity recorded in the cell-attached mode were shown in Fig. 3. At 16.6 mM glucose concentration, the continuous spike potentials resembling action potential for insulin secretion were detected and the channel activity was nearly abolished in the control beta cells. In the diabetic beta cells, however, the inhibitory effects were lacking and the spike potentials hardly appeared.

As illustrated in Fig. 4, glyceraldehyde (2 mM) inhibited K_{ATP} channel activity completely in both the control and NIDDM rats. The difference of its inhibitory potency between the two groups was negligible. The spike potentials were detected at 5 mM of glyceraldehyde in both groups.

Fig. 5 and 6 show dose-dependence of the inhibition of K_{ATP} channel activity by dihydroxyacetone (DHA, 2~10 mM). The inhibitory effect of DHA on K_{ATP} channel activity was smaller in NIDDM group (Fig. 5). In particular, the differences were significant between the control and NIDDM rats at 5 and 10 mM of DHA (Fig. 6).

Fig. 7 shows the inhibitory effect of a representative reactive disulfide, 2,2'-dithio-bis (5-nitropyridine) (DTBNP) on K_{ATP} channel of NIDDM beta cell. On addition of DTBNP (50 μM) in the bath solution, there was a

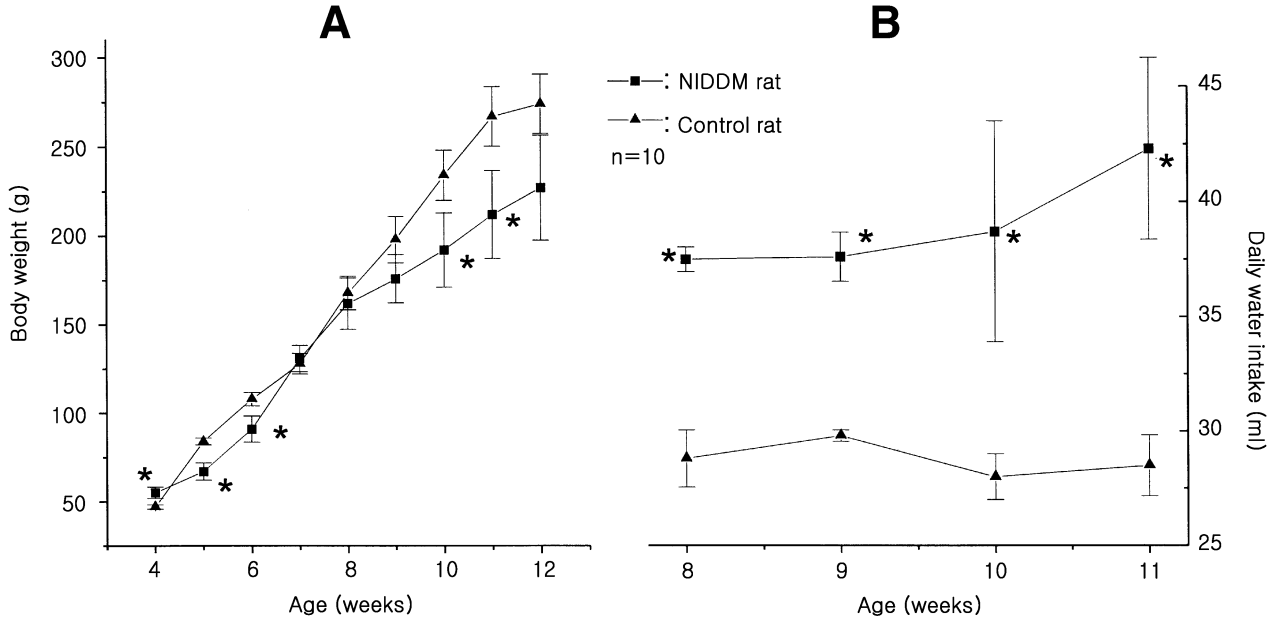

Fig. 2. Differences in body weight gain(A) and daily water intake(B) between control and NIDDM rats. * $p<0.05$ compared to values of control in each age. Mean±SE.

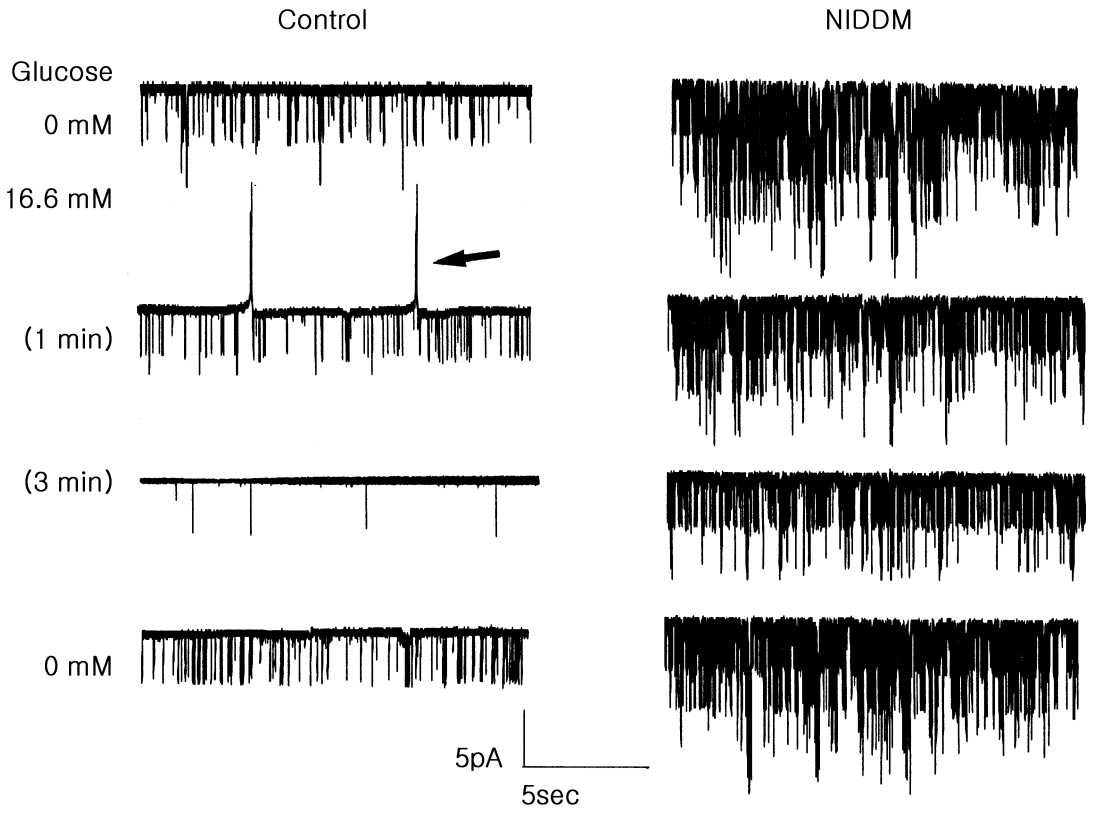

Fig. 3. Effects of glucose on K_{ATP} channel activity in β-cells of control and NIDDM rats. Cell-attached mode, V_p=0 mV. Spike potentials(arrow) evoked by glucose(16.6 mM) are detected in controls.

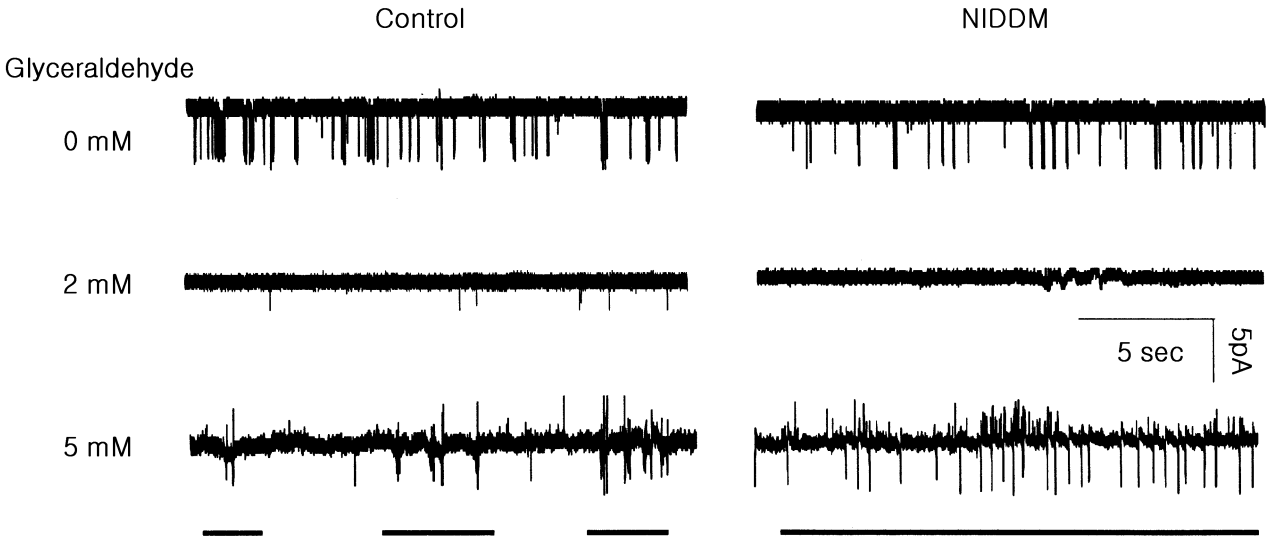

Fig. 4. Effects of glyceraldehyde on K_{ATP} channel activity in β-cells of control and NIDDM rats. Cell-attached mode, Vp=0 mV. Spike potentials are indicated by bar in both groups.

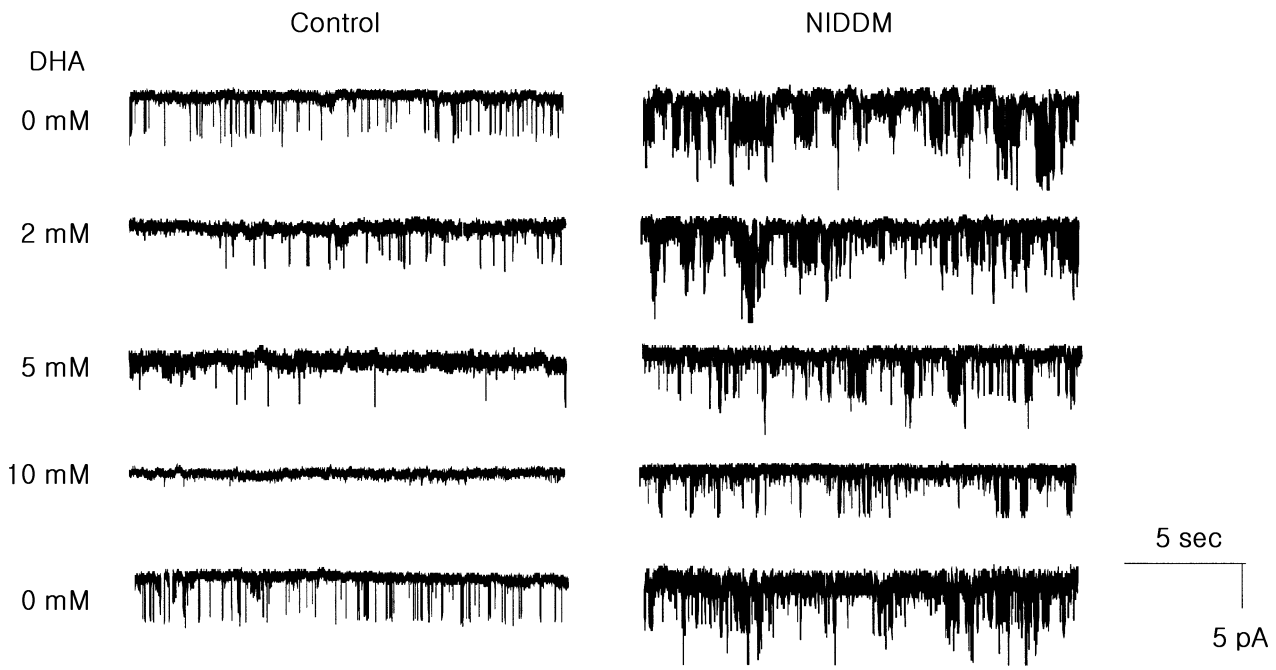

Fig. 5. Representative K_{ATP} channel currents record on β-cells of control and NIDDM rats when concentrations of dihydroxyacetone (DHA) are increased. Cell-attached mode, V_p=0 mV.

rapid reduction in K_{ATP} channel activity. The inhibitory effect of DTBNP was also reversed by 2 mM dithiothreitol (DTT), a disulfide reducing agent.

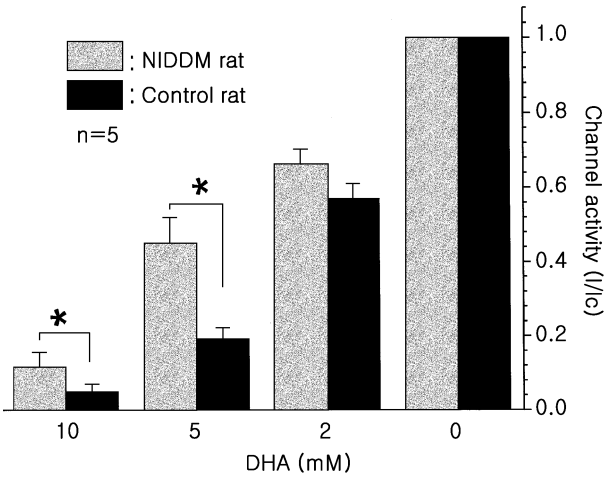

Fig. 6. Effects of dihydroxyacetone(DHA) on K_{ATP} channel activity in β-cells of control and NIDDM rats when concentrations of DHA are increased. * $p<0.05$. Mean±SE.

DISCUSSION

The major physiological stimulus for insulin release from the pancreatic beta cell is an increase in the plasma glucose concentration (10). In response to stimulatory glucose concentrations, the membrane of the beta cell depolarizes, initiating a characteristic pattern of electrical activity through the voltage-dependent Ca^{2+} channels (11). The initial depolarization for insulin secretion results from a decrease in K_{ATP} channel activity of the beta cell which occurs as a consequence of glucose metabolism (12). The primary determinant of K_{ATP} channel activity in the beta cell is the intracellular ATP (13). Therefore, we may hypothesize that ATP produced by glucose metabolism inhibits K_{ATP} channel activity.

Insulin response to glucose and inhibitory effect of glucose on K_{ATP} channel activity are markedly impaired in patients with NIDDM. There is considerable evidence that the reduced sensitivity to glucose of the diabetic beta cell is not due to alterations in the properties of K_{ATP} channel per se. Tsuura et al. (6) demonstrated that the sensitivity of K_{ATP} channels to intracellular ATP in the beta cells of NIDDM rats is virtually identical to that in the control beta cells. As also shown in this experiment, the effect of sulfhydryl oxidation by DTBNP on K_{ATP} channels in the diabetic beta cells was similar

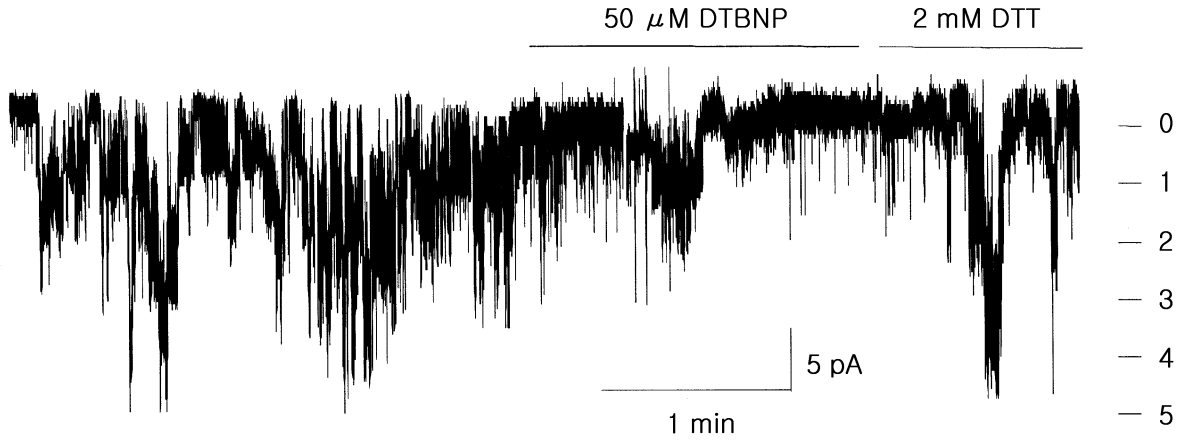

Fig. 7. Effect of 2,2'-dithio-bis(5-nitropyridine)(DTBNP) on K_{ATP} channel activity in β-cell of NIDDM rat. Cell-attached mode, V_p=0 mV. Addition of DTBNP(50 μM) almost totally blocked channel activity.

to that in the normal beta cells which is demonstrated by Islam et al. (14). These findings suggest that the reduced sensitivity to glucose is due to the decrease in ATP production caused by impaired glucose metabolism in the diabetic beta cells.

The glyceraldehyde sensitivity of K_{ATP} channels was not distinguishable between the beta cells of control and NIDDM rats in this study. A similar result was obtained by a genetic rodent model of NIDDM (GK rats, 15), indicating that the step responsible for the disturbed ATP production may not exist in the distal portion of glycolysis after glyceraldehyde-3-phosphate. Tu and Tuch (16) recently concluded that the inability of the fetal rat beta cell to secrete insulin in response to glucose could not be explained by the immaturity of glucokinase or the glycolytic pathway. This suggests the proximal steps of glucose metabolism are not related to defects in NIDDM.

In the present study, the inhibitory effect of DHA on K_{ATP} channel activity was found to be lower in the beta cells of NIDDM rats compared to controls. Furthermore, some K_{ATP} channels in the beta cells of NIDDM rats had not been inhibited at all by DHA (data not shown). Since the properties of the K_{ATP} channel in NIDDM rats have been shown not to be altered, the reduced sensitivity of DHA on the K_{ATP} channel activity would be due to impaired utilization of DHA for intracellular ATP production.

The conversion of DHA to DHA-phosphate by triokinase does not seem probable because triokinase activity has been reported to be abundantly present in islets of GK rats (17). The most likely site responsible for decreased glucose metabolism is thought to be a malfunction of the glycerol phosphate shuttle where DHA-phosphate enters. During the glycolytic degradation of the hexose, NADH is produced in the cytosol at the step catalyzed by glyceraldehyde phosphate dehydrogenase. Because accumulation of the reducing equivalents inhibits glycolysis, NADH produced in the cytosol must be reoxidized. It has been demonstrated that pancreatic islets are equipped with hydrogen shuttles that can transport reducing equivalents into mitochondria where oxidation takes place (18, 19). The glycerol phosphate shuttle is one of such shuttles.

Several reports demonstrated impairment of the glycerol phosphate shuttle in a rodent model of NIDDM (20, 21). The activity of mitochondrial glycerol phosphate dehydrogenase (mGPDH), which is known to be the key enzyme in this shuttle, was found to be lower in NIDDM rats than in normal controls (22). Ishihara et al. (23), however, reported that mGPDH abundance in insulin secreting cell lines (MIN6 and HIT cells) is not directly related to their insulin secretory capacity in response to glucose, and reduced expression of mGPDH is not the primary cause of abnormal insulin secretory responses in HIT cells as a diabetic beta cell model. They hypothesized NADH supply might be rate limiting in the situation that mGPDH was adequately expressed. Since tumoral cell lines may not be identical to native beta cells with respect to the relative importance of the glycerol phosphate shuttle among several shuttles, these data should be treated with caution. The regulation of reducing equivalent transfer and that of glucose metabolism appeared to be mediated by the shuttle mechanisms that operate in concert. Further studies are needed to elucidate these complex interrelated regulatory mechanisms.

In conclusion, DHA can inhibit K_{ATP} channel activity

of pancreatic beta cells. In NIDDM rats, however, the sensitivity of DHA on K_{ATP} channels was reduced, presumably due to the insufficient utilization of DHA for intracellular ATP production. These results are consistent with the hypothesis that the glycerol phosphate shuttle is functionally impaired in NIDDM beta cells.

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