Alterations in Basal and Glucose-stimulated Voltage-dependent Ca^{2+} Channel Activities in Pancreatic β Cells of Non–Insulin-dependent Diabetes Mellitus GK rats

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Abstract

In genetically occurring non–insulin-dependent diabetes mellitus (NIDDM) model rats (GK rats), the activities of L- and T-type Ca^{2+} channels in pancreatic β cells are found to be augmented, by measuring the Ba^{2+} currents via these channels using whole-cell patch-clamp technique, while the patterns of the current-voltage curves are indistinguishable. The hyper-responsiveness of insulin secretion to nonglucose depolarizing stimuli observed in NIDDM β cells could be the result, therefore, of increased voltage-dependent Ca^{2+} channel activity.

Perforated patch-clamp recordings reveal that the augmentation of L-type Ca^{2+} channel activity by glucose is markedly less pronounced in GK β cells than in control β cells, while glucose-induced augmentation of T-type Ca^{2+} channel activity is observed neither in the control nor in the GK β cells. This lack of glucose-induced augmentation of L-type Ca^{2+} channel activity in GK β cells might be causatively related to the selective impairment of glucose-induced insulin secretion in NIDDM β cells, in conjunction with an insufficient plasma membrane depolarization due to impaired closure of the ATP-sensitive K^+ channels caused by the disturbed intracellular glucose metabolism in NIDDM β cells. (*J. Clin. Invest.* 1996. 97:2417–2425.) Key words: Ca^{2+} channel • GK rat • pancreatic β cell • patch clamp

Introduction

One of the characteristics of non–insulin-dependent diabetes mellitus (NIDDM),[1] the selective impairment of glucose-induced insulin secretion from pancreatic β cells is found both in patients with NIDDM (1) and in NIDDM animal models, including neonatally streptozotocin-induced diabetic (NSZ) (2) and genetically diabetic GK rats (3). In contrast, nonglucose depolarizing stimuli such as arginine have been found to elicit rather enhanced insulin responses in these subjects (1–3). However, the molecular basis of these paradoxical phenomena has not yet been fully clarified.

The ATP generated through intracellular glucose metabolism in β cells is known to depolarize the plasma membrane by inhibiting ATP-sensitive K^+ channels (K_{ATP} channels), thereby eliciting Ca^{2+} entry via voltage-dependent Ca^{2+} channels (4). It is also known that glucose augments the activity of L-type Ca^{2+} channels by its metabolism in pancreatic β cells (5). We have demonstrated previously that the electrophysiological properties of ATP-sensitivity of K_{ATP} channels are not altered in β cells of GK rats, a genetic model of NIDDM, while the glucose-sensitivity of K_{ATP} channels is markedly impaired (6, 7). Therefore, the impairment of glucose-induced insulin release might be the result of reduced glucose metabolism, but not of alterations of the K_{ATP} channels in diabetic β cells. Likewise, the hyperresponsiveness (or relative preservation) of insulin release to nonglucose depolarizing stimuli in NIDDM cannot be attributed to alterations of the K_{ATP} channels in β cells. The alternative hypothesis that intracellular Ca^{2+} ($[Ca^{2+}]_i$) signaling distal to the K_{ATP} channels, including Ca^{2+} channel activity, might be hyperactive in diabetic β cells thus urged us to explore the electrophysiological properties of voltage-dependent Ca^{2+} channels and $[Ca^{2+}]_i$ dynamics after depolarization in NIDDM β cells.

In the present study, we have recorded L- and T-type voltage-dependent Ca^{2+} channel activities in β cells of GK rats, using the whole-cell patch-clamp technique together with measurement of $[Ca^{2+}]_i$ by dual-wave spectrofluorometry. Augmentation of L- and T-type voltage-dependent Ca^{2+} channel activities by intracellular glucose metabolism in β cells of GK rats was also assessed, using the perforated patch-clamp technique.

Methods

Characterization of animals. Male GK rats were donated by Takeda Pharmaceutical Research Laboratories (Osaka, Japan). Age-matched male Wistar rats also were obtained as controls. The animals were fed on standard lab chow ad libitum and allowed free access to water in an air-conditioned room with a 12-h light/dark cycle until used for the experiments. Since histological abnormalities are known to appear in islet cells of GK rats from 4 mo of age (8), they were used at 8–12 wk of age. The body wt of GK rats (266±16 [mean±SE] g, n = 16) was not statistically different from that of controls (293±14 g, n = 16). Blood samples were collected via the inferior vena cava immediately before isolation of the pancreatic islets, under pentobarbital anesthesia (40 mg/kg body wt). The plasma glucose concentration in the fed state, measured by the glucose-oxidase method, was 21.5±0.83 mM

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1. *Abbreviations used in this paper:* $[Ca^{2+}]_i$, intracellular Ca^{2+}; Cp, capacitance; K_{ATP} channel, ATP-sensitive K^+ channel; NIDDM, non–insulin-dependent diabetes mellitus.

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($n = 16$) in GK and 10.0±0.38 mM ($n = 13$) in control rats, respectively ($P < 0.001$).

In some cases, whole pancreatic tissues were fixed in 10% formalin, embedded in paraffin and sectioned. Insulin-positive islet cells were identified by stain, using indirect peroxidase-conjugated immunohistological staining for insulin, as reported previously (9).

Assessment of insulin secretory capacity. Insulin secretory capacity was measured by the batch incubation method, using islets freshly isolated by collagenase digestion, according to the method reported (6). Briefly, the islets were preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate buffer (KRBB) supplemented with 3.3 mM glucose and 0.2% BSA, and then incubated in batches of ~ 10 islets for 30 min at 37°C in 0.7 ml of KRBB containing 0.2% BSA and a given concentration of glucose and/or 30 mM K^+. The amount of released insulin was determined by RIA using rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as the standard (6). Experiments using the same protocol were repeated at least three times to ascertain reproducibility.

Dispersion of pancreatic islets and culture of single islet cells. Isolated islets were washed in PBS and incubated with 0.25% trypsin and 1 mM EDTA solution (GIBCO BRL, Grand Island, NY) for 3 min at 37°C. Digestion was terminated by rinsing the cells in cold PBS containing 0.6 mg/ml soybean trypsin inhibitor (Sigma, St. Louis, MO). Dispersed islet cells were suspended in RPMI 1640 medium supplemented with 10% FBS. They were cultured on small glass coverslips (15 × 4 mm) overnight at 37°C using RPMI 1640 medium supplemented with 10% FBS in humidified air containing 5% CO_2. Individual coverslips were transferred to the test chamber and placed on an inverted microscope for electrophysiological experiments.

In some experiments, the dispersed islet cells were used fresh rather than being cultured, to exclude the possibility of alterations in cellular properties during culture. In these cases, glass coverslips coated with Cell-Tak® (Collaborative Biomedical Products, Bedford, MA) were used to accelerate cell adhesion, and the experiments were completed within 4 h after cell dispersion.

In patch-clamp and fura-2 experiments, pancreatic β cells were identified by their larger size (10) and confirmed by the criteria described by Pressel and Misler (11).

Patch-clamp study. Whole-cell current recordings were made in β cells at 25–27°C with a patch-clamp amplifier (EPC-9; HEKA Elektronik, Lambrecht, Germany). If not specifically mentioned, cultured β cells were used for current acquisition. Data currents were filtered at 3 kHz, and digitalized signals were stored for analysis by an on-line computer (Macintosh, Cupertino, CA) using Pulse and Pulse-fit® program (HEKA Elektronick, Lambrecht, Germany).

Patch pipettes (tip resistance: 2–4 megaohms) were pulled from borosilicate glass capillaries, coated with Sylgard® (Dow Corning, Midland, MI), fire-polished, and filled with a pipette solution containing (in mM) 130 CsCl, 10 tetraethylammonium chloride (TEA-Cl), 5 $MgCl_2$, 4 ATP-Na_2, 0.4 GTP-Na_2, 10 EGTA, and 10 Hepes (pH 7.2 with CsOH), as reported previously (12). Gigaohm seal was made in normal extracellular solution (138 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 3.3 glucose, 10 Hepes, pH 7.4). After formation of whole-cell mode, Ba^{2+} currents were recorded in a Ba^{2+}-containing bath solution composed of (in mM) 30 $BaCl_2$, 116 CsCl, 10 TEA-Cl, 1.2 $MgCl_2$, and 10 Hepes (pH 7.4 with CsOH). Total exposure time of the β cells to the glucose-free bathing solution did not exceed 5 min. Leak current subtraction was not performed; however, only cells with minimum leaks were adopted for current acquisition, the input resistance of which should be greater than 2 gigaohms in Ba^{2+}-containing bath solution.

Ba^{2+} currents via L-type Ca^{2+} channels were measured by applying depolarizing step pulses (between −40 mV and +50 mV in 10 mV steps, of 500 ms duration at 0.5 Hz) from a holding potential (V_h) of −45 mV.

Ba^{2+} currents via T-type Ca^{2+} channels were recorded according to a double-pulse protocol, in which 100-ms command pulses (between −40 mV and +40 mV in 10 mV steps) are applied after 500-ms prepulses to −100 mV from a V_h of −45 mV. These prepulses can release T-type Ca^{2+} channels from inactivation (13). To eliminate L-type Ca^{2+} channel current components, Ba^{2+} currents elicited without applying the prepulse were subtracted from the corresponding ones with the prepulses.

Perforated patch-clamp recordings were performed using pipette solution composed of (in mM) 125 CsCl, 5 $MgCl_2$, 20 sucrose, 1 EGTA, and 10 Hepes (pH 7.2 with CsOH). Nystatin was solubilized by detergent action of fluorescein according to the method of Yawo and Chuma (14). The final concentration of nystatin was adjusted to 100 μg/ml. Voltage-clamp current recordings were started after the series resistance fell to < 40 megaohms and remained stable. For evaluation of L-type Ca^{2+} channels, cells were held at V_h of −70 mV, and Ca^{2+} channel currents were elicited by 500-ms step pulses to 0 mV. T-type Ca^{2+} channel currents under perforated patch-clamp also were assessed by a double pulse protocol equivalent to that for conventional whole-cell recordings (see above), although only one command pulse of −10 mV was applied. Effects of ambient glucose on L and T-type Ca^{2+} channel activities were studied after 4-min exposure to 16.7 mM glucose.

[^{3}H]nitrendipine binding study. The number of specific binding sites for [^{3}H]nitrendipine was assessed according to the method previously described (12), using freshly dispersed islet cells. Binding sites expressed as moles per cell were converted to the number of actual sites by multiplying by Avogadro constant.

Measurement of intracellular Ca^{2+}. 1 μM fura-2 AM (Molecular Probes Inc., Eugene, OR) was loaded in freshly dispersed islet cells for 30 min at 37°C. A heat-controlled chamber on a stage of an inverted microscope kept at 36±1°C was superfused with Krebs-Ringer-bicarbonate-Hepes buffer (KRBH) composed of (in mM) 130 NaCl, 5.2 KCl, 2.8 $CaCl_2$, 1.3 KH_2PO_4, 1.6 $MgCl_2$, 20 $NaHCO_3$, 3.3 glucose, and 10 Hepes, pH 7.4. Ratiometry of the emission light (510 nm) elicited by dual wave excitation light (340 and 360 nm) was performed on an ARGUS-100 image analyzing system (Hamamatsu Photonics, Hamamatsu, Japan). In vivo calibration was done according to the equation:

$$[Ca^{2+}]_i = K_m (R - R_{min})/(R_{max} - R),$$

where $K_m = 224$ nM, R_{min} and R_{max} have their usual meanings (15).

Results were expressed as mean±SE. Statistical significance was evaluated by unpaired and paired Student's t test, and $P < 0.05$ was considered significant.

Results

Insulin secretory capacity of GK islets. Insulin was secreted dose dependently in the control islets in response to glucose: a sig-

Table I. Insulin Secretory Capacity of the Islets Obtained from GK and Control Rats

	control rat ($n = 6$)	GK rat ($n = 6$)
	μU/islet/30 min	*μU/islet/30 min*
3.3 mM glucose	15.8±0.8	22.0±1.9*
11.1 mM glucose	32.5±4.3§	30.2±2.8§
16.7 mM glucose	80.5±4.6‖¶	34.5±3.9‡§
30 mM K^+ with 11.1 mM glucose	60.6±7.3¶	117.5±13.4‡¶

*Significant at $P < 0.05$ vs. corresponding control. ‡Significant at $P < 0.01$ vs. corresponding control. §Significant at $P < 0.05$ vs. 3.3 mM glucose. ‖Significant at $P < 0.01$ vs. 3.3 mM glucose. ¶Significant at $P < 0.01$ vs. 11.1 mM glucose.

Table II. Percentage of Insulin Release by 30 mM K^+ to that by Normal K^+ in the Presence of 3.3 and 16.7 mM Glucose

	control rat (n = 6)	GK rat (n = 6)
	%	%
3.3 mM glucose	261±13	455±67*
16.7 mM glucose	112±9‡	316±66*

*Significant at $P < 0.05$ vs. corresponding control. ‡Significant at $P < 0.01$ vs. 3.3 mM glucose.

nificant increase was observed not only between 3.3 and 11.1 mM, 3.3 and 16.7 mM, but also between 11.1 and 16.7 mM glucose stimuli. On the other hand, in the GK islets, 16.7 mM glucose failed to elicit a significantly larger insulin secretion than did 11.1 mM glucose. Insulin secretion stimulated by 16.7 mM glucose was impaired significantly in the GK islets, compared to the controls, while basal insulin secretion induced by 3.3 mM glucose was significantly higher in the GK islets. With 11.1 mM glucose, the insulin secretory capacities of both groups were similar. However, high K^+-induced insulin secretion in the presence of 11.1 mM glucose was significantly larger in the GK islets (Table I).

In another series of experiments, the insulinotropic effect of high K^+-depolarization were examined with either 3.3 or 16.7 mM glucose. The percentage of released insulin in high K^+ solutions to that in normal K^+ counterparts was compared between GK and its control islets (Table II). The effects of high K^+-depolarization were prominent in the GK islets at both 3.3 and 16.7 mM glucose.

β cell population in islets. The β cell population in the GK islets (71.0±2.1%, n = 18) was not statistically different from that in the control islets (72.1±1.3%, n = 18) (Fig. 1).

[^{3}H]nitrendipine binding sites. The number of specific binding sites for [^{3}H]nitrendipine per islet cell was not statistically different between the GK group (1,480±240, n = 6) and the control group (1,750±209, n = 6).

Ba^{2+} currents via L-type Ca^{2+} channels. Sustained inward currents were recorded when 500-ms depolarizing pulses of > -30 mV were applied from a holding potential of −45 mV in cultured β cells both in control and in GK rats (Fig. 2, *A* and *B*, *upper traces*). These currents were maximal at about +10 mV, and decreased with the shifting of the command pulse level to more positive potentials. Although the membrane capacitance (Cp) of the GK β cells (5.57±0.27 pF, n = 19) was not statistically different from that of the control β cells (5.57±0.20 pF, n = 19), the L-type Ca^{2+} channel currents recorded from the GK β cells (Fig. 2 *B*, *upper trace*) were mark-

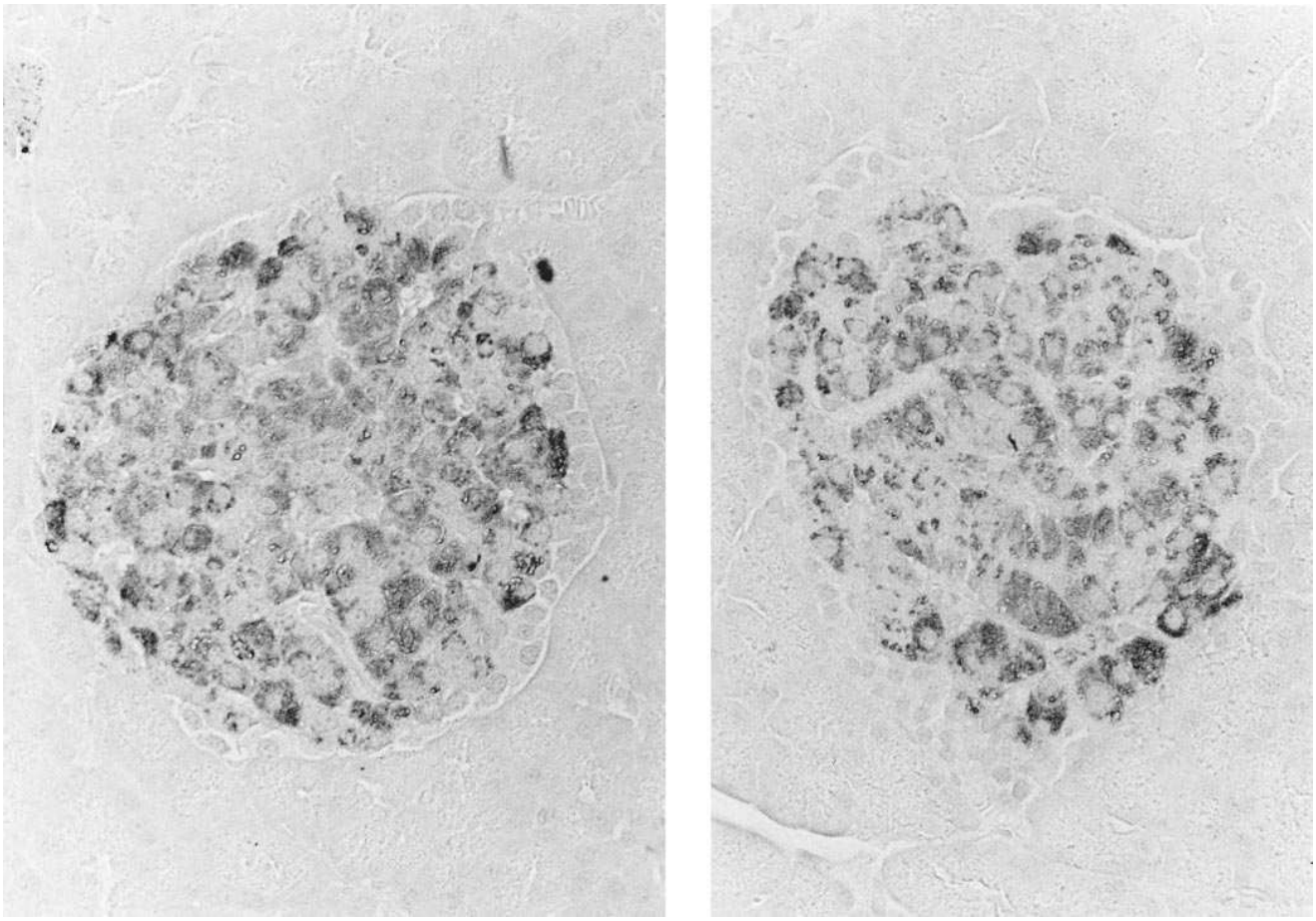

Figure 1. Comparison of β cell population in the pancreatic islets between the GK (*left*) and the control (*right*) islets by immunohistological staining for insulin-positive cells.

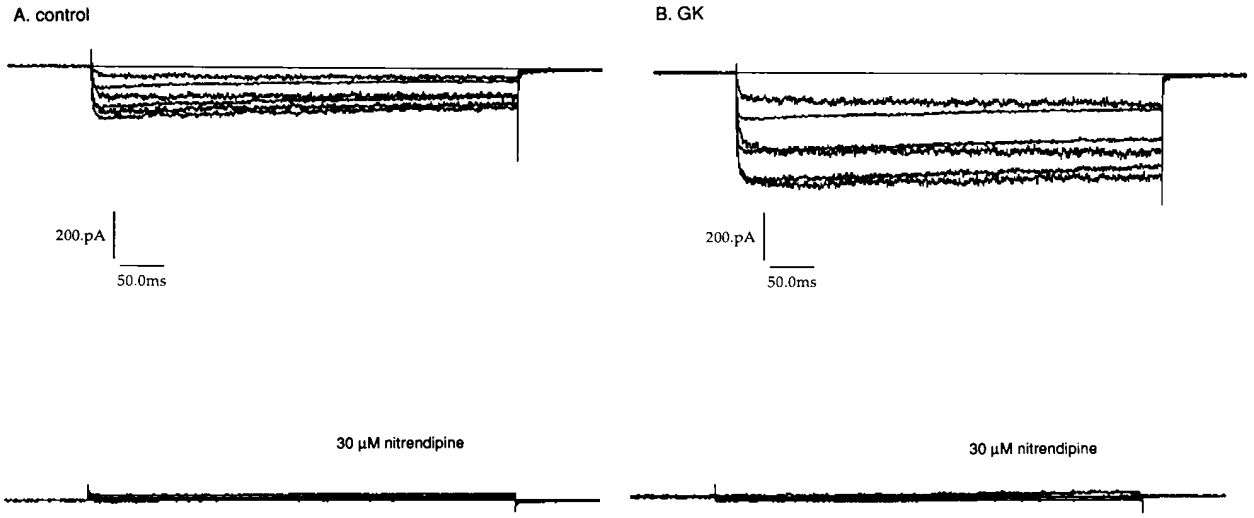

Figure 2. Representative whole-cell Ba^{2+} currents via L-type Ca^{2+} channels in the cultured pancreatic β cells. Depolarizing step pulses (to −40 ∼ +50 mV) were applied from the holding potential of −45 mV. (*A*) control β cell (Cp = 7.03 pF). (*B*) GK β cell (Cp = 5.60 pF). Sustained inward currents were elicited in both groups (*upper traces*) which were inhibited by 30 μM nitrendipine (*lower traces*).

edly greater than those from the control β cells (Fig. 2 *A*, *upper trace*). These currents were completely inhibited by adding 30 μM nitrendipine to the bath solution (Fig. 2, *A* and *B*, *lower traces*). The activation time course of the L-type Ca^{2+} channel currents were nicely fitted by single exponentials (Fig. 3, *A* and *B*) in the depolarization range of −20 mV ∼ +40 mV, and the activation time constants (τ_a) were the same in both groups (Fig. 3 *C*).

When the current densities, calculated by dividing the current by the membrane capacitance, were plotted against voltage, similar bell-shaped I-V curves were obtained in cultured β cells of both control and GK rats (Fig. 4 *A*). The current densities in the GK β cells were significantly larger than those in controls at −20 to +40 mV. Similar results were obtained in freshly dispersed β cells (Fig. 4 *B*).

Ba^{2+} currents via T-type Ca^{2+} channels. As shown in Fig. 5, T-type Ca^{2+} channel components were assessed by double-pulse protocol. Transient inward currents were recognized after subtraction of L-type components. The T-type Ca^{2+} channel currents in the GK β cells (Fig. 5 *B*) were greater than those in the controls (Fig. 5 *A*). Bell shaped I-V curves were obtained in both groups, and current densities in the GK β

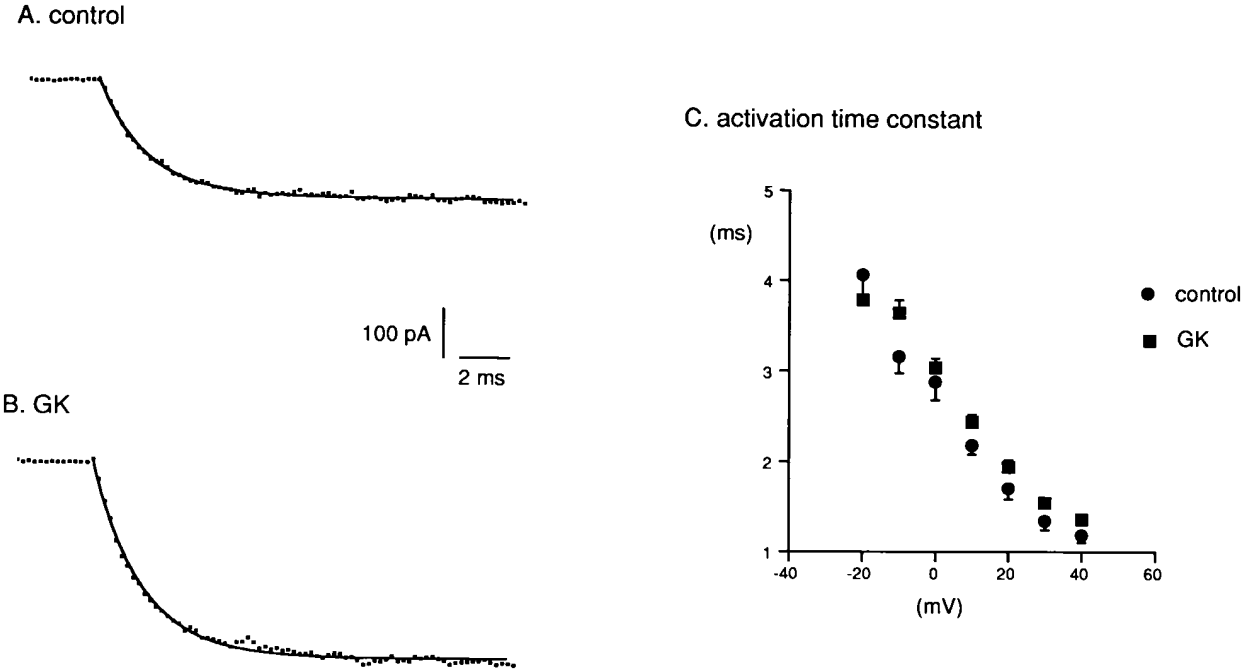

Figure 3. Activation time course of L-type Ca^{2+} channel currents. (*A*) control β cell (Cp = 5.78 pF). (*B*) GK β cell (Cp = 5.80 pF). The L-type Ca^{2+} channel currents elicited by the command pulses between −20 mV and +40 mV could be fitted by single exponentials, although no difference of activation time constants (τ_a) was observed in either group (*C*).

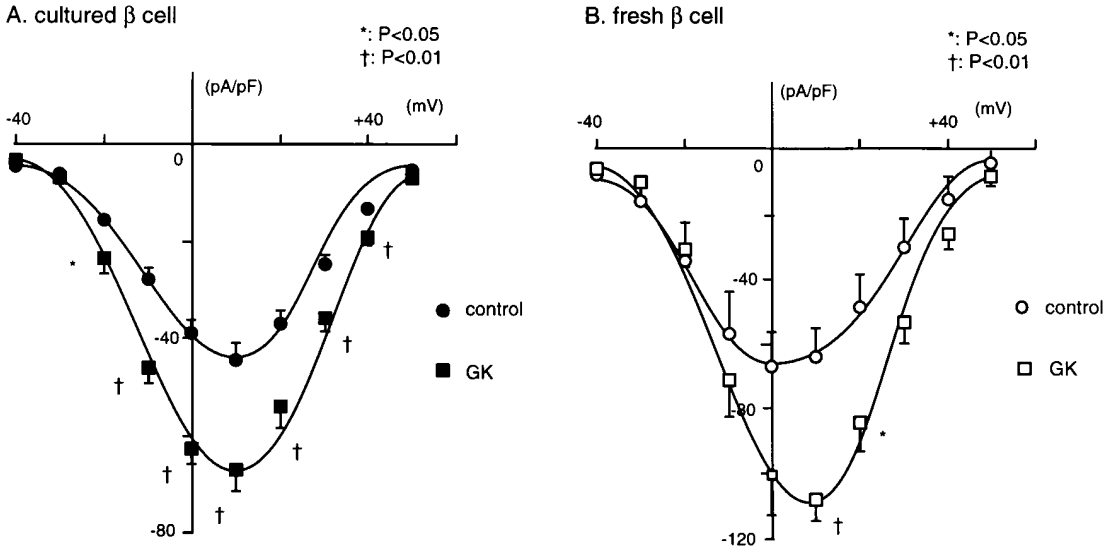

Figure 4. I-V curves for L-type Ca^{2+} channel currents in the β cells from GK and control rats. The current densities were plotted against depolarization potentials. (*A*) I-V curves for the cultured β cells. Filled circles and squares indicate the current densities for the control β cells ($n = 19$) and for the GK β cells ($n = 19$), respectively. (*B*) I-V curves for the freshly isolated β cells. Open circles represent current densities in the control β cells ($n = 5$), and open squares in the GK β cells ($n = 5$). The current densities were significantly larger in the GK group than in those in controls at $^{*}P < 0.05$ and $^{\dagger}P < 0.01$.

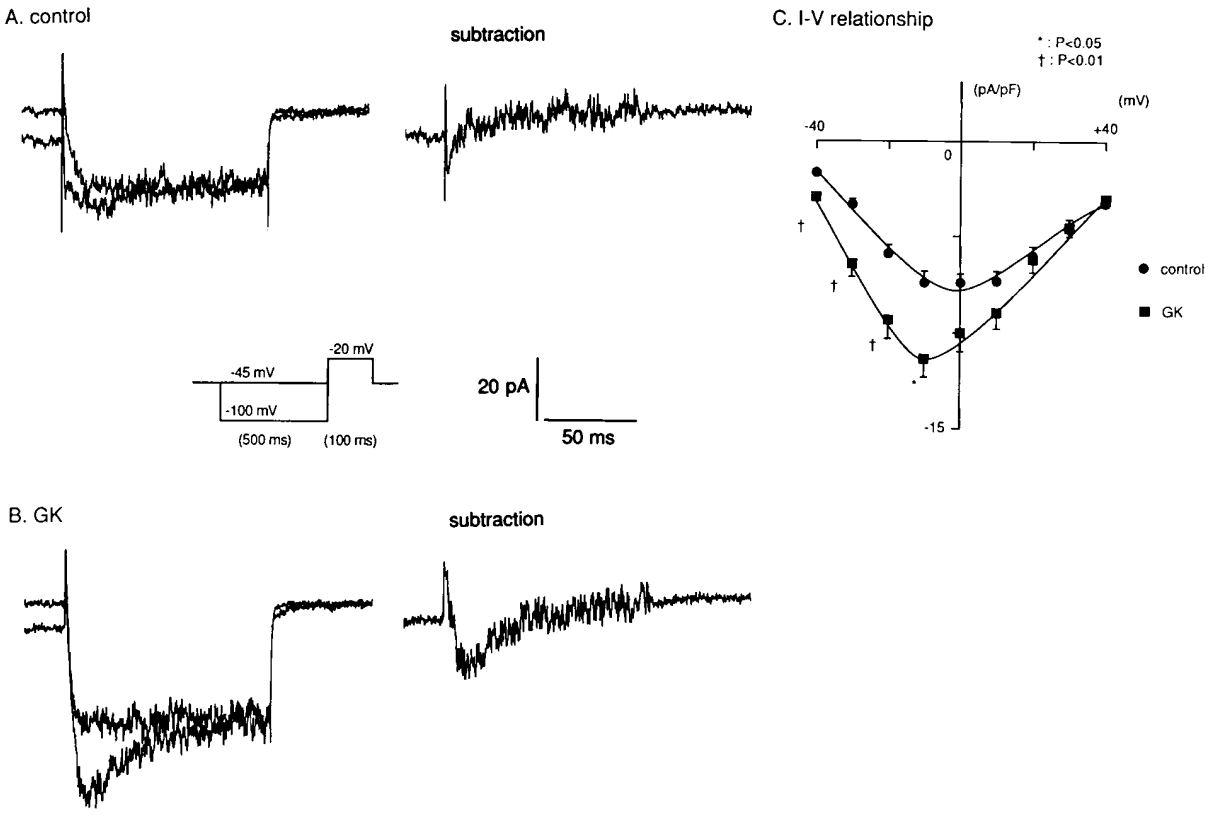

Figure 5. T-type Ca^{2+} channel currents assessed by double pulse protocols. T-type Ca^{2+} channel currents were obtained by subtracting the L-type components in the absence of prepulses from the combined L- and T-type Ca^{2+} channel currents in the presence of −100 mV prepulses in the control β cells (*A*, Cp = 6.40 pF) and in the GK β cells (*B*, Cp = 6.31 pF). (*C*) I-V curves of the T-type Ca^{2+} channel currents. The current densities were significantly greater in the GK β cells (*closed square*, $n = 13$) than in the control β cells (*closed circle*, $n = 16$) at $^{*}P < 0.05$ and $^{\dagger}P < 0.01$.

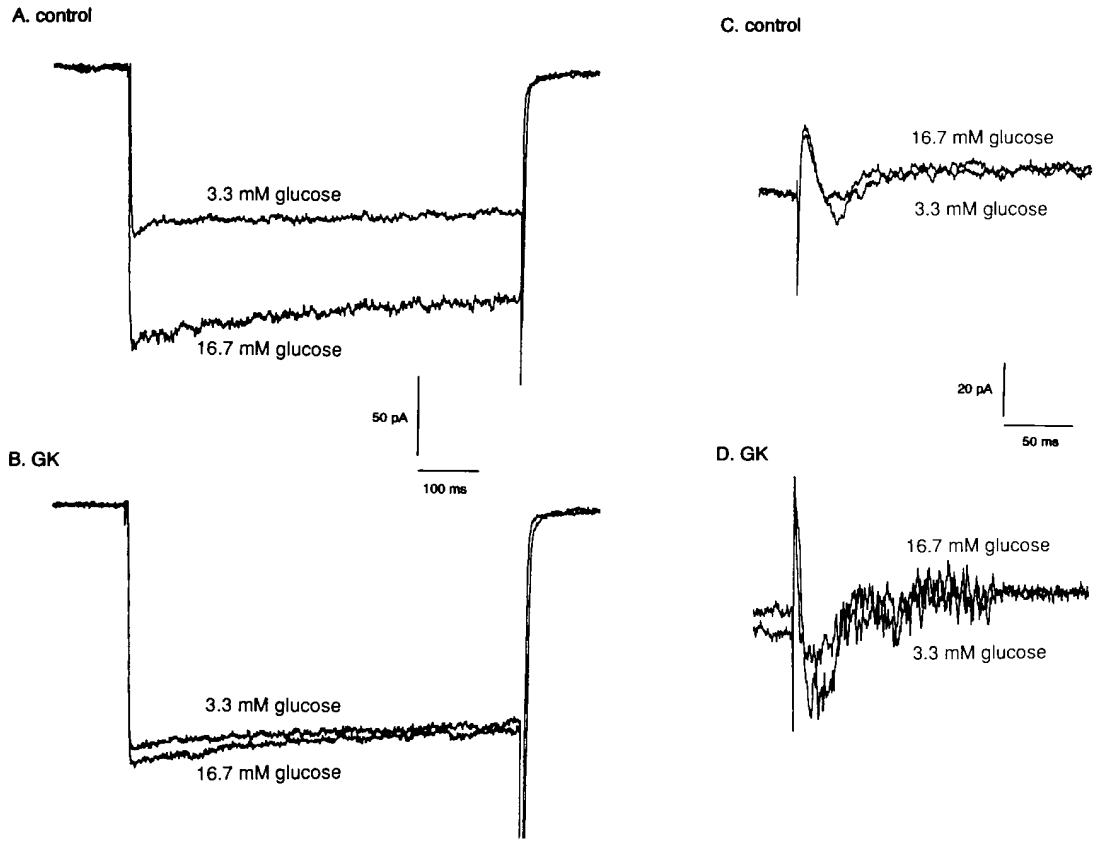

Figure 6. Effects of glucose on Ca^{2+} channel currents recorded under perforated patch-clamp configuration. (*A*) L-type Ca^{2+} channel currents were recorded first in 3.3 mM ambient glucose and then after 4-min exposure to 16.7 mM glucose, by applying 500-ms depolarization pulses to 0 mV from the holding potential of −70 mV in the control β cell (*upper traces*, Cp = 5.80 pF) and in the GK β cell (*lower traces*, Cp = 5.21 pF). (*B*) T-type Ca^{2+} channel currents were recorded with 3.3 and 16.7 mM glucose by applying double-pulse protocols in the control (*upper traces*, Cp = 5.74 pF) and the GK (*lower traces*, Cp = 5.70 pF) β cells.

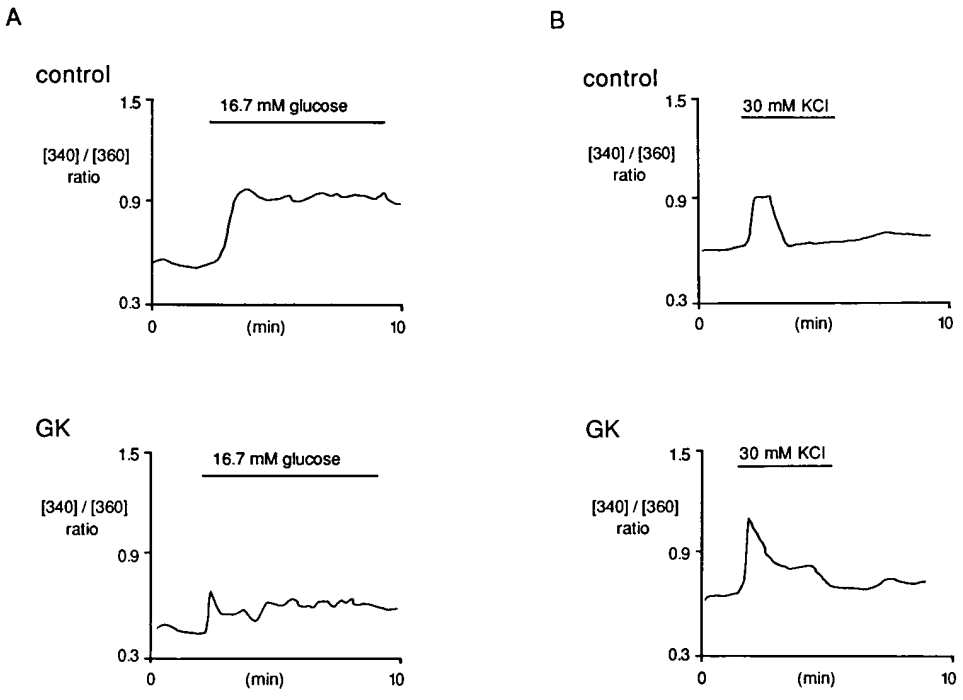

Figure 7. The representative traces of the $[Ca^{2+}]_i$ responses to 16.7 mM glucose (*A*) and 30 mM K^+ (*B*) in the control (*top*) and GK β cells (*bottom*). The vertical axis is the ratio of the fluorescence elicited by 340 and 360 nm excitation lights.

cells were significantly larger than in the controls at −40 to −10 mV (Fig. 5 *C*).

Modulation of Ca^{2+} channel activation by glucose, assessed by perforated-patch recording. Using perforated patch-clamp technique, the modulation of L-type Ca^{2+} channel activation by ambient glucose was investigated. 4-min exposure to 16.7 mM glucose augmented Ca^{2+} channel currents in the control β cells (Fig. 6 *A*). Glucose was less effective on the Ca^{2+} channels in the GK β cells (Fig. 6 *B*). In the control β cells, peak current amplitude after glucose exposure was significantly larger (442±39 pA, $n = 6$, $P < 0.05$) than before (280±20 pA). In the GK β cells, no significant change was observed in the peak current amplitude before (386±12 pA, $n = 5$) and after (381±13 pA) glucose challenge. Currents at 3.3 mM glucose were significantly larger in GK β-cells than in controls ($P < 0.05$).

On the other hand, 16.7 mM glucose did not increase T-type Ca^{2+} channel currents (Fig. 6, *C* and *D*). Peak currents after high glucose exposure (GK: 42.9±4.5 pA, $n = 6$, control: 26.9±4.3 pA, $n = 5$) were not significantly different from the corresponding currents before high glucose exposure (GK: 47.5±3.3 pA, control: 30.0±5.4 pA).

$[Ca^{2+}]_i$ response in β cells. Application of 16.7 mM glucose induced intracellular Ca^{2+} oscillations or Ca^{2+} plateaus in the control β cells. However, only transient peaks or small oscillations were observed in the GK β cells (Fig. 7 *A*). Maximal Ca^{2+} concentrations as well as incremental changes in $[Ca^{2+}]_i$ in response to glucose were significantly reduced in the GK β cells (Table III). On the other hand, depolarization induced by 30 mM K^+ at 3.3 mM glucose elicited prompt and large Ca^{2+} peaks in both groups (Fig. 7 *B*). The peak Ca^{2+} concentrations and incremental changes in $[Ca^{2+}]_i$ were somewhat greater in the GK β cells than in the controls. However, when 30 mM K^+ was applied with 16.7 mM glucose, the $[Ca^{2+}]_i$ responses were comparable between the GK and the control β cells (Table III).

Using β cells hyperpolarized by 200 μM diazoxide, a known K_{ATP} channel opener, the functional modulation of voltage-dependent Ca^{2+} channels by intracellular glucose metabolism was examined by monitoring $[Ca^{2+}]_i$ dynamics (16, 17). After exposure of the β cells to either 3.3 or 16.7 mM glucose for 3 min, the degree of $[Ca^{2+}]_i$ increase in response to depolarization by 30 mM K^+ was assessed. As shown in Table IV, the initial peak $[Ca^{2+}]_i$ after high K^+-induced depolarization was significantly augmented after exposure to 16.7 mM glucose, compared to that of 3.3 mM glucose in the control β cells, but this effect was lacking in the GK β cells.

Table III. $[Ca^{2+}]_i$ Response to 16.7 mM Glucose and 30 mM K^+ in the β Cells Obtained from the GK and the Control Rats

control rat (nM)		GK rat (nM)	
normal K^+		normal K^+	
3.3 mM glucose	16.7 mM glucose	3.3 mM glucose	16.7 mM glucose
46±4	257±20	39±4	114±11*
(increment: 211±19, $n = 47$)		(increment: 75±8,* $n = 42$)	
3.3 mM glucose		3.3 mM glucose	
normal K^+	30 mM K^+	normal K^+	30 mM K^+
50±4	224±12	59±5	435±49*
(increment: 175±12, $n = 53$)		(increment: 376±45,* $n = 47$)	
normal K^+	30 mM K^+	normal K^+	30 mM K^+
3.3 mM glucose	16.7 mM glucose	3.3 mM glucose	16.7 mM glucose
50±3	459±13	56±3	438±15
(increment: 409±11, $n = 35$)		(increment: 382±13, $n = 29$)	

*Significant at $P < 0.01$ vs. corresponding control.

Table IV. Comparison of $[Ca^{2+}]_i$ Change in Response to 30 mM K^+ Depolarization in the β Cells Hyperpolarized by 200 μM Diazoxide between GK and Control Rats

control rat (nM)		GK rat (nM)	
3.3 mM glucose		3.3 mM glucose	
normal K^+	30 mM K^+	normal K^+	30 mM K^+
12±13	112±13	20±4	180±19*
(increment: 100±12, $n = 16$)		(increment: 153±10,* $n = 12$)	
16.7 mM glucose		16.7 mM glucose	
normal K^+	30 mM K^+	normal K^+	30 mM K^+
13±4	273±44	10±4	163±22
(increment: 260±41, $n = 18$)		(increment: 159±17,* $n = 13$)	

*Significant at $P < 0.05$ vs. corresponding control.

Discussion

The present study shows for the first time that both L- and T-type Ca^{2+} channel activities are increased in β cells of GK rats, a genetically occurring NIDDM model. This finding is compatible with observations that Ca^{2+} channel activities are enhanced in β cells of NSZ rats, which we have recently reported (12). Increased activity of Ca^{2+} channels, therefore, seems to be a common phenomenon of NIDDM status, irrespective of etiology, where both the selectively impaired insulin secretion in response to glucose and the augmented insulin response to nonglucose depolarizing stimuli are observed (2, 3).

The increased activity of the voltage-dependent Ca^{2+} channels might be a putative mechanism of the augmented insulin response to depolarizing stimuli other than glucose, which frequently has been observed in both human NIDDM and its animal models (2, 3, 12). Since the $[Ca^{2+}]_i$ response to depolarization induced by high K^+ was found to be greater in GK β cells than in controls, the observed augmentation of the insulinotropic action of high K^+ could be due to the increased Ca^{2+} channel activity upon depolarization. On the other hand, the higher basal insulin secretion in the GK islets might not be due to the increased Ca^{2+} channel activity, since no difference was observed in the basal $[Ca^{2+}]_i$ level between the two groups.

A lack of glucose-induced augmentation of L-type Ca^{2+} channel activity is indicated by the perforated patch-clamp findings in the present study of pancreatic β cells of GK rats. The lack of enhancement of high K^+-induced $[Ca^{2+}]_i$ elevation by glucose in diazoxide-treated β cells of GK rats accords with this. The impaired glucose-sensitivity of L-type Ca^{2+} channels, therefore, could be a factor in the selective impairment of glucose-induced $[Ca^{2+}]_i$ elevation in NIDDM β cells. Glucose has been shown to control insulin secretion from β cells by three major mechanisms: closure of K_{ATP} channels by ATP generated by glucose metabolism (4), modulation of L-type Ca^{2+} channel activity through glucose metabolism (5), and undefined pathways independent of K_{ATP} channel closure (17). The impaired insulin response to glucose in NIDDM β cells, there-

fore, might seem to be a combined consequence of the failure by glucose to augment L-type Ca^{2+} channels and the insufficient depolarization due to the decreased glucose sensitivity of K_{ATP} channel closure (6), both of which are attributable to reduced glucose metabolism after acute glucose challenge in β cells of GK rats (Fig. 8).

The enhanced L-type Ca^{2+} channel activities in GK rats are probably due not to an increased number of L-type Ca^{2+} channels in diabetic β cells, since the number of specific binding sites for [^{3}H]nitrendipine per cell was found to be the same in the GK and control groups. Because the β cell population in the GK islets was comparable to that in the controls, the number of nitrendipine binding sites of total islet cells should reflect those of the β cells of both groups. This suggests that the greater whole-cell L-type Ca^{2+} channel currents in GK β cells might be explained by the augmented open probability of the channels, the magnified single-channel conductance, or both. Direct measurements of single-channel properties are needed to fully clarify the mechanism of increased Ca^{2+} channel activities. Since the neuroendocrine type α_1 subunit of the L-type Ca^{2+} channel has many putative phosphorylation sites, mainly in cytoplasmically located COOH-terminal regions (18), the altered state of their phosphorylation under conditions of prolonged hyperglycemia in NIDDM might be linked to changes in the single-channel properties of diabetic β cells. One hypothesis is that the L-type Ca^{2+} channel activities in GK β cells, having undergone full augmentation due to functional alterations of the intracellular signaling system in the diabetic state (19, 20), cannot be further increased. This is compatible with the results in the present perforated patch-clamp study of the GK β cells, which show L-type Ca^{2+} channel currents to be augmented at a low glucose level but not any longer by acute glucose challenge.

T-type Ca^{2+} channel activities also were found to be increased in the GK β cells. T-type Ca^{2+} channel currents in rats were found to be relatively small, as previously reported (13), and the role of T-type Ca^{2+} channel currents on insulin secretory mechanisms in rodents remains unclear.

The pathophysiological influence of long-term increase in both L- and T-type Ca^{2+} channel activities on NIDDM β cells deserves concern. There is evidence of chronic overload of intracellular Ca^{2+} in some tissues, such as myocardium and vascular smooth muscle cells in human NIDDM patients and its animal models, and it has been discussed in conjunction with progressive cytotoxicity (21, 22). A recent study (23) has shown that Ca^{2+} channel activities in pancreatic β cells are enhanced by application of sera obtained from IDDM patients and that the enhancement of Ca^{2+} influx induces programmed cell death (apoptosis) of β cells (24). Since sustained hyperglycemia under NIDDM conditions could continuously stimulate Ca^{2+} entry into β cells through the hyperfunctioning Ca^{2+} channels, and since other nonglucose depolarizing stimuli such as positively charged amino acids and sulfonylureas, the oral hypoglycemic agents, also can induce intracellular Ca^{2+} overload, increased Ca^{2+} channel activities might be a cause of the gradual loss of intact β cell populations in diabetic islets.

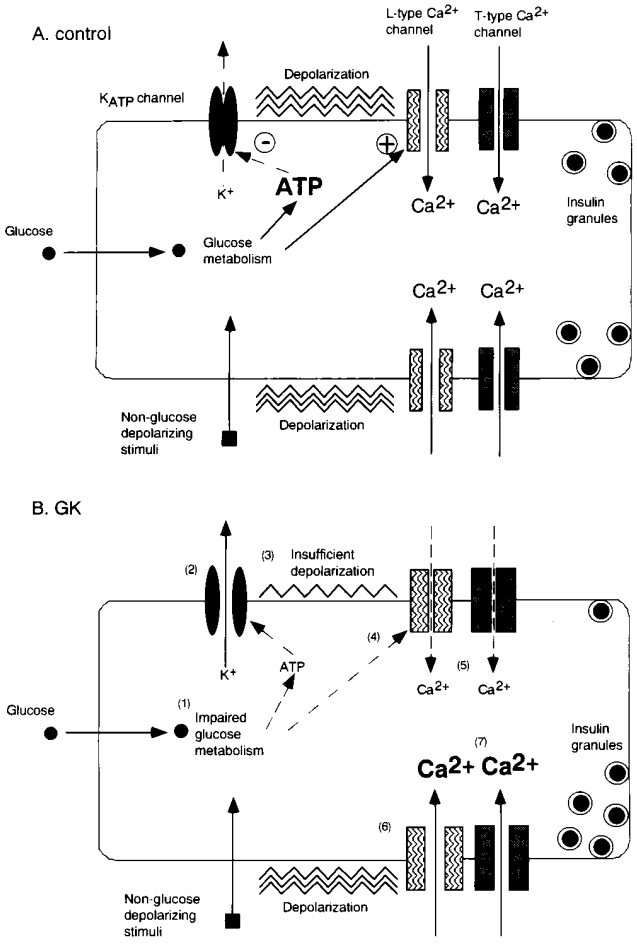

Figure 8. Schematic representation of the pathophysiological changes in the GK β cell (*B*) compared to its normal counterpart (*A*). In the GK β cell, ATP production is reduced because of the impaired glucose metabolism (*1*), resulting in the poor closure of K_{ATP} channels (*2*). Insufficient depolarization (*3*) is not able to activate voltage-dependent Ca^{2+} channels. In addition, direct modulation of L-type Ca^{2+} channels by intracellular glucose metabolism is also diminished (*4*), leading to the impaired elevation of $[Ca^{2+}]_i$ (*5*). On the other hand, when nonglucose depolarizing stimuli are applied, the GK β cells can depolarize enough to open voltage-dependent Ca^{2+} channels, the activities of which are enhanced in the GK β cells (*6*). Large Ca^{2+} influxes through the activated voltage-dependent Ca^{2+} channels may be related to the supranormal insulin release to these stimuli (*7*).

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