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Delayed Ca^{2+} Response to Glucose in Diabetic GK Rat

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The spontaneously diabetic non-obese GK (Goto-Kakizaki) rat exhibits high basal plasma glucose and insulin levels and poor glucose-induced insulin secretion, which makes it a suitable model for non-insulin dependent diabetes mellitus, NIDDM. The aim of this study was to investigate the handling of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$), the key regulator of insulin secretion, in GK rat single pancreatic islets. For this purpose the influence of high glucose (16.7 mM) and arginine (20 mM) on $[Ca^{2+}]_i$ was studied in GK and Wistar rat islets, which served as controls. The data obtained suggest that glucose which through its metabolism generates ATP needed for closure of the K_{ATP} channels and membrane depolarization, induces a delayed $[Ca^{2+}]_i$ response in the GK rat pancreatic islet. This delay in $[Ca^{2+}]_i$ response is likely to result from a defective metabolism of glucose in the diabetic islet.

One feature of non-insulin dependent diabetes mellitus (NIDDM) is the impairment of glucose-induced insulin release from the pancreatic β-cell (1-3). Animal models of diabetes may help in elucidating the mechanisms responsible for such an impairment. The spontaneously diabetic Goto-Kakizaki (GK) rat is an interesting model of β-cell impairment (4-7). It was shown that glucose metabolism in GK rat islets is altered, with diminished ratio between oxidation and utilization of glucose and increased glucose cycling (8). Pancreatic β-cell from GK rats also possess a deficient oxidative metabolism of glucose in their mitochondria (9), the step responsible for metabolic perturbation being located in the glycerol phosphate shuttle (10-12). Interestingly, the pancreatic islet of GK rat also demonstrated some underexpression of GLUT2 glucose transporter which, however, could not explain the profound reduction in glucose-induced insulin secretion (13). It was observed that elevation in cAMP concentration markedly enhanced insulin release in GK rat but not in control rat (14). Studies in permeabilized pancreatic islets showed higher Ca^{2+}-induced insulin release in GK rats than in controls (15,16). In studies which used GK rat as a spontaneous model for NIDDM, the information about the function of one of the key regulators of insulin secretion namely cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, is rather limited. In one study (9), however, there was no difference in bulk glucose-induced 90 min uptake of $^{45}Ca^{2+}$ between islets of GK and nondiabetic Wistar rats. In another study, alterations in voltage-dependent Ca^{2+} channel activity were shown in dispersed pancreatic β-cells of GK rat (17).

In the present study we investigated how glucose and arginine affected $[Ca^{2+}]_i$ in single GK rat pancreatic islets. Arginine induces insulin release by causing β-cell membrane depolarization (18) and as a consequence opening of voltage-gated L-type Ca^{2+} channels (18-20).

METHODS

Animals and islet isolation procedure. Diabetic GK rats were obtained from our local colony initiated in August 1989 with five pairs of GK rats (F_{40}) issued from the original colony established by Goto and Kakizaki (4) (Tohoku University School of Medicine, Sendai, Japan). Non-diabetic Wistar rats (B & K Universal, Sollentuna, Sweden) were used as control animals. Animals were maintained under conditions described earlier (8) and pancreatic islets were isolated aseptically by collagenase digestion (21) and cultured overnight at 37 °C in the presence of 11 mM glucose, or when indicated at 5.5 mM glucose, and 10% heat-inactivated newborn calf serum (Flow Laboratories, Irwine, U.K.) (8).

Measurements of $[Ca^{2+}]_i$. In perifusion experiments, a medium supplemented with 1 mg/ml bovine serum albumin containing (in mM): NaCl 125, KCl 5.9, $CaCl_2$ 1.3, $MgCl_2$1.2, HEPES 25 and pH 7.4, with different concentrations of glucose and other additions, was used. Average sized islets were chosen for experiments. Islets were loaded with 2 μM fura 2/AM for 1 hour in medium containing 3.3 mM glucose. After loading, a single islet was transferred to an open perifusion chamber custom built for microscopic work and maintained at a temperature of 37 °C. Measurements of 340 / 380 nm fluorescence ratio (R), reflecting $[Ca^{2+}]_i$, were done as previously described (22). A flow rate of 150 μl/ min was selected to be optimal not to perturb the islet in our system. The time for complete change

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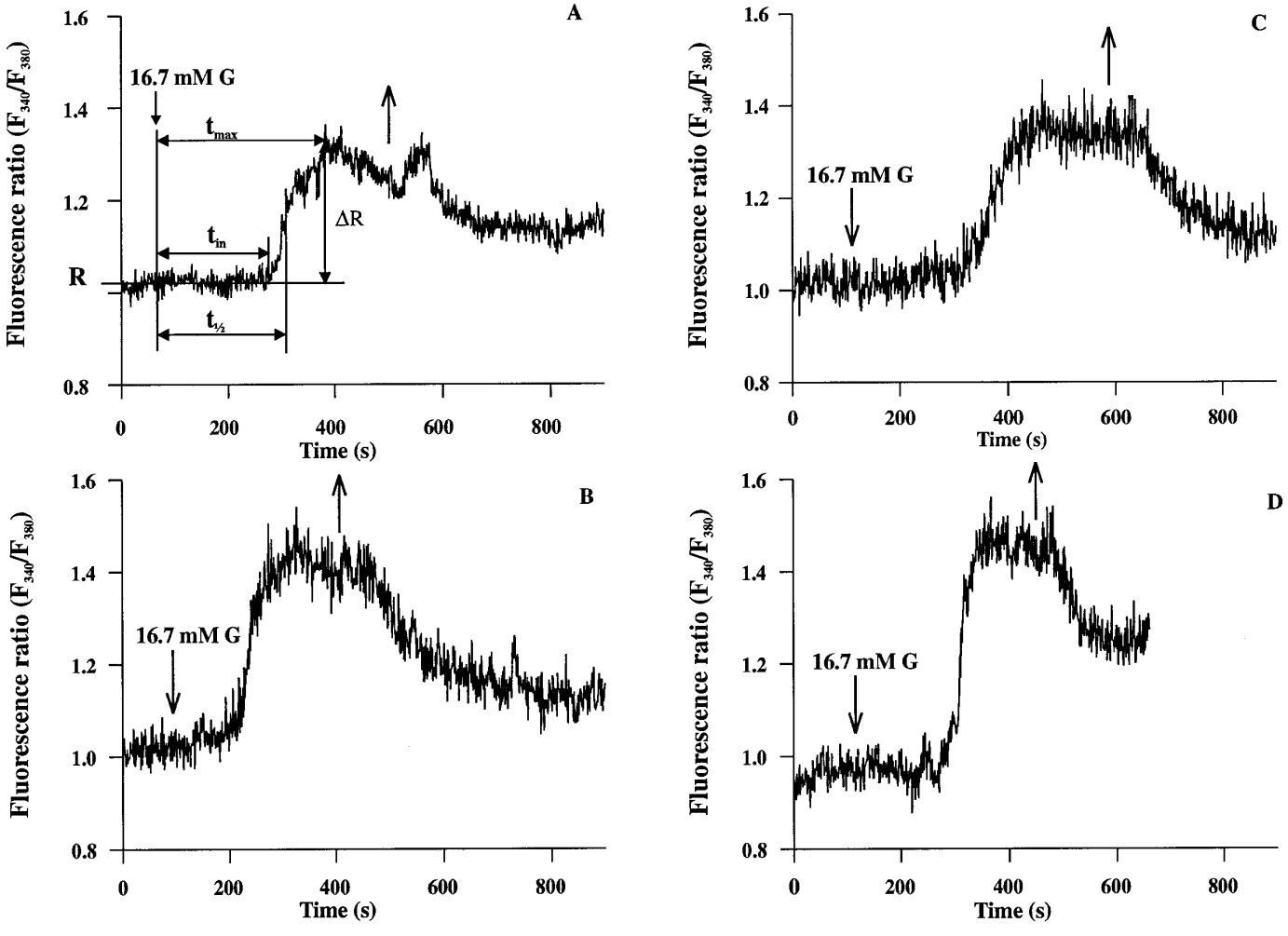

FIG. 1. Comparison of glucose-induced changes in $[Ca^{2+}]_i$ in single GK (A,C) and Wistar (B,D) rat pancreatic islets. Islets were cultured at 11 mM (A,B) or 5.5 mM (C,D) glucose overnight. 16.7 mM glucose was added after preperifusion with a buffer containing 3.3 mM glucose. Addition and withdrawal of 16.7 mM glucose (G) are shown by arrows. Each of the curves shown is representative of results of 9-14 separate experiments, performed under the same conditions in islets from the different animals. Quantitative analysis of changes in $[Ca^{2+}]_i$ were made according to the figure. Abbreviations used are as follows: R, 340 / 380 nm fluorescence ratio, reflecting $[Ca^{2+}]_i$, at basal conditions (3.3 mM glucose); ΔR, $[Ca^{2+}]_i$ response after stimulation; t_{in}, lag time of the $[Ca^{2+}]_i$ response; $t_{1/2}$, the time corresponding to ΔR/2; t_{max}, the time of the maximal $[Ca^{2+}]_i$ response.

of solution in the area near the islet was 44 s after switching of media. This delay was corrected in the graphical representations.

For a quantitative comparison of the $[Ca^{2+}]_i$ responses in GK rat and Wistar rat islets the parameters t_{in}, $t_{1/2}$, t_{max}, R and ΔR (see Fig. 1A) were chosen.

Statistical analysis. Data analysis was done using the program Sigma Plot for Windows (version 1.02, Jandel Corp., San Rafael). Results are expressed as mean ± SEM, and significance of differences was assessed by Student's t-test for unpaired data.

RESULTS

Influence of 16.7 mM glucose stimulation on $[Ca^{2+}]_i$ in islets from GK and Wistar rats. In the GK rat islets the rate of increase in $[Ca^{2+}]_i$, after addition of 16.7 mM glucose, was significantly slower compared to that in Wistar rat islets (Fig.1, Table 1). One of the reasons was a delayed lag time for $[Ca^{2+}]_i$ response (t_{in}). Basal $[Ca^{2+}]_i$ level as well as the magnitude of increases in $[Ca^{2+}]_i$ (ΔR) were not significantly different between GK rat and Wistar rat islets.

Effects of culture conditions on the responses of GK rat and Wistar rat islets to 16.7 mM glucose. The data on $[Ca^{2+}]_i$ in response to 16.7 mM glucose in GK rat and Wistar rat islets, cultured at 5.5 mM glucose overnight, are shown in Fig.1 and Table 1. In comparison to culture at 11 mM glucose, culture at 5.5 mM glucose overnight gave rise to a more delayed $[Ca^{2+}]_i$ response to 16.7 mM glucose in both GK rat and Wistar rat islets. The difference in kinetic parameters of $[Ca^{2+}]_i$ responses to 16.7 mM glucose between GK rat and

TABLE 1

$[Ca^{2+}]_i$ Response Pattern in Diabetic GK and Nondiabetic Wistar (Control) Rat Pancreatic Islets, Incubated Overnight at Different Concentrations of Glucose

	GK rat						Wistar rat					
	n	t_{in} (s)	$t_{1/2}$ (s)	t_{max} (s)	R	ΔR	n	t_{in} (s)	$t_{1/2}$ (s)	t_{max} (s)	R	ΔR
Incubated overnight in 11 mM glucose												
Glucose (16.7 mM)	14	162 ± 19*	218 ± 15*	288 ± 166**	0.91 ± 0.08	0.30 ± 0.05	11	107 ± 19	161 ± 16	222 ± 16	1.02 ± 0.12	0.30 ± 0.05
Arginine (20 mM), glucose (3.3 mM)	10	39 ± 7	69 ± 6	118 ± 8	0.90 ± 0.03	0.24 ± 0.02*	7	39 ± 10	65 ± 11	117 ± 9	0.93 ± 0.04	0.17 ± 0.02
Incubated overnight in 5.5 mM glucose												
Glucose (16.7 mM)	9	304 ± 19***¶	419 ± 17***¶	517 ± 22***¶	1.04 ± 0.14	0.27 ± 0.04	9	175 ± 10‡	229 ± 11‡	289 ± 12‡	1.05 ± 0.13	0.30 ± 0.04

* $P < 0.05$ GK vs Wistar rats
** $P < 0.01$ GK vs Wistar rats
*** $P < 0.001$ GK vs Wistar rats
¶ $P < 0.001$ incubated in 5.5 mM vs 11 mM glucose overnight, stimulation by 16.7 mM glucose (GK rats)
‡ $P < 0.01$ incubated in 5.5 mM vs 11 mM glucose overnight, stimulation by 16.7 mM glucose (Wistar rats)

Wistar rat islets were even more pronounced for islets cultured overnight at 5.5 mM glucose. The magnitudes of $[Ca^{2+}]_i$ increases to 16.7 mM glucose were not significantly different between GK rat and Wistar rat islets incubated at 5.5 mM glucose overnight. There was no significant difference in the magnitudes of $[Ca^{2+}]_i$ responses to 16.7 mM glucose between islets incubated at 5.5 mM or 11 mM glucose overnight.

Arginine effect on $[Ca^{2+}]_i$ response in GK rat pancreatic islets. Addition of arginine (20 mM) to the medium containing 3.3 mM glucose did not change the kinetic parameters of $[Ca^{2+}]_i$ response (Fig. 2, Table 1). Nevertheless, the magnitude of increase in $[Ca^{2+}]_i$ from the basal level was significantly higher ($P<0.05$) in GK rat islets in comparison to controls.

DISCUSSION

This study focused on changes in $[Ca^{2+}]_i$ in diabetic GK rat islets which possessed decreased glucose-induced insulin secretion. The reason for the specific interest in $[Ca^{2+}]_i$ is due to the fact that this ion has a well-known key regulatory role in insulin secretion under physiological conditions (19,20,22). The problem tackled put several questions into consideration. First, are diabetic GK rat islets, possessing impairment in glucose-induced insulin release, also having disturbances in $[Ca^{2+}]_i$ changes in response to the sugar. If such an impairment indeed exists, is it then connected to events following membrane depolarization, e.g. activity of Ca^{2+} channels, or to some sites in the metabolic pathway for glucose.

In this study we demonstrate that in the GK rat, a spontaneous rat model of NIDDM, β-cell $[Ca^{2+}]_i$ response to glucose is impaired. The latter impairment consisted of a delayed response in $[Ca^{2+}]_i$ in GK rat islets compared to control Wistar rat islets, especially clear in islets cultured at 5.5 mM glucose. This protracted $[Ca^{2+}]_i$ response in GK rat islets corresponded not only to reduced insulin release in batch incubation studies (5,8,9,14), but also to the delayed initiation of glucose-stimulated insulin secretion as seen in the perifused islets of GK rats (23). The data obtained are also in agreement with an impaired response to glucose seen in pancreatic β-cells of streptozotocin-induced NIDDM rats (24). In β-cells of a streptozotocin-induced rat model of NIDDM (24) and in GK rat dispersed β-cells (17), a decrease in the magnitude of glucose stimulated $[Ca^{2+}]_i$ response (ΔR), in comparison to Wistar rat b-cells, was found. Such a decrease could not be found in whole islets from GK rat in the present study.

The stimulus-secretion coupling for glucose in β-cells involves ATP formation due to glucose metabolism, which leads to closure of K_{ATP} channels, membrane depolarization, opening of L-type voltage-gated Ca^{2+}-channels, increase in $[Ca^{2+}]_i$, and subsequently exocytosis of insulin (25). Any defect in this cascade of events, prior to the change in $[Ca^{2+}]_i$, may contribute to the impaired $[Ca^{2+}]_i$ as well as insulin responses in the GK rat islet. To clarify whether the impaired rise in $[Ca^{2+}]_i$ in the GK rat islet could be linked to events distal to closure of K_{ATP} channels, i.e. membrane depolarization, experiments with arginine (20 mM) were performed. Electrogenic transport of this amino acid depolarizes the β-cell membrane (18). Data on increased $[Ca^{2+}]_i$ in response to 20 mM arginine in the presence of 3.3 mM glucose in islets from GK rat, in comparison to control, suggest that the influx of Ca^{2+} through the voltage-gated L-type Ca^{2+} channels, if anything, is more active in the diabetic islets. These results coincide with the elevated $[Ca^{2+}]_i$ response in β-cells of streptozotocin-induced diabetic rats (24) and are in agreement with an augmentation of the activity of voltage-gated Ca^{2+} channels in dispersed pancreatic β-cells of GK rats (17).

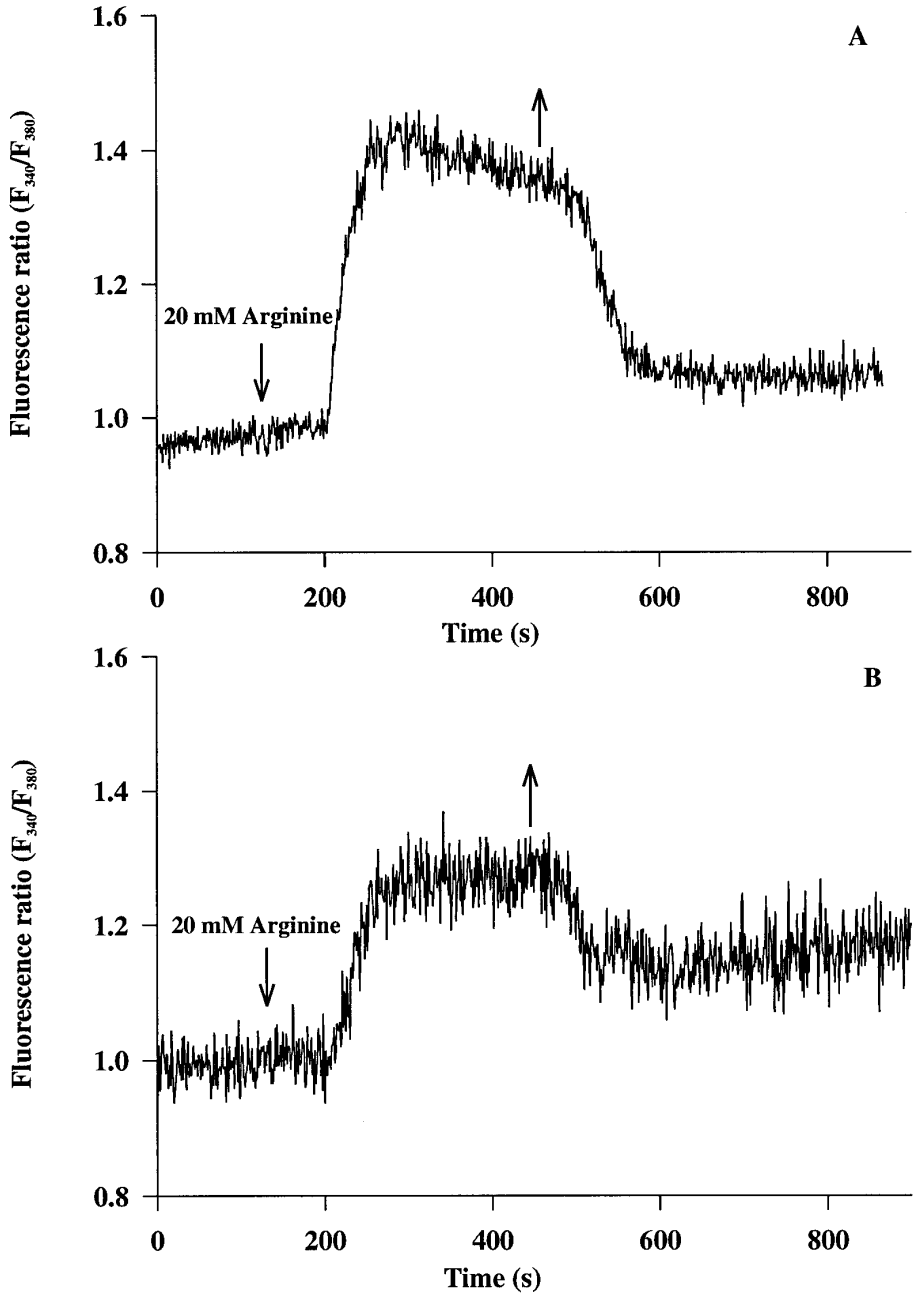

FIG. 2. Effect of 20 mM arginine in the presence of 3.3 mM glucose on $[Ca^{2+}]_i$ in single GK (A) and Wistar (B) rat pancreatic islets. Addition and withdrawal of 20 mM arginine are shown by arrows. Each of the curves shown is representative of results of 7-10 separate experiments, performed under the same conditions in islets from the different animals.

What has been discussed so far, suggest that a key impairment in glucose-induced increase in $[Ca^{2+}]_i$ in GK rat islets is localized proximal to membrane depolarization and opening of voltage-gated Ca^{2+}-channels, most likely reflecting an impaired glucose metabolism.

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