Defective glucose-dependent cytosolic Ca\(^{2+}\) handling in islets of GK and nSTZ rat models of Type 2 diabetes

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Abstract

We examined to what extent the abnormal glucose-dependent insulin secretion observed in NIDDM (non-insulin-dependent diabetes mellitus) is related to alterations in the handling of cytosolic Ca\(^{2+}\) of islets of Langerhans. Using two recognized rat models of NIDDM, the GK (Goto–Kakizaki) spontaneous model and the nSTZ (neonatal streptozotocin) induced model, we could detect several common alterations in the glucose-induced [Ca\(^{2+}\)]\(_i\), cytosolic responses. First, the initial reduction of [Ca\(^{2+}\)]\(_i\), following high glucose (16–7 mM) observed routinely in islets obtained from non-diabetic Wistar rats could not be detected in GK and nSTZ islets. Second, a delayed response for glucose to induce a subsequent 3% increase of [Ca\(^{2+}\)]\(_i\), over basal level was observed in both GK (321 ± 40 s, \(n = 11\)) and nSTZ (326 ± 38 s, \(n = 13\)) islets as compared with Wistar islets (198 ± 20 s, \(n = 11\)), values representing means ± S.E.M. Third, the rate of increase in [Ca\(^{2+}\)]\(_i\) in response to a high glucose challenge was 25% and 40% lower in GK and nSTZ respectively, as compared with Wistar islets. Fourth, the maximal [Ca\(^{2+}\)]\(_i\), level reached after 10 min of perifusion with 16–7 mM glucose was lower with GK and nSTZ islets and represented respectively 60% and 90% of that of Wistar islets. Further, thapsigargin, a blocker of Ca\(^{2+}\)-ATPases (SERCA), abolished the initial reduction in [Ca\(^{2+}\)]\(_i\), observed in response to high glucose and induced fast [Ca\(^{2+}\)]\(_i\) oscillations with high amplitude in Wistar islets. The latter effect was not seen in GK and nSTZ islets. In these two NIDDM models, several common alterations in glucose-induced Ca\(^{2+}\) handling were revealed which may contribute to their poor glucose-induced insulin secretion.


Introduction

Prior to the triggering of insulin exocytosis from beta cells exposed to high glucose, a number of metabolic and ionic changes occurs leading to an increase in the cytoplasmic concentration of Ca\(^{2+}\) or [Ca\(^{2+}\)]\(_i\). The main pathway involves the closure of ATP-sensitive K\(^+\) channels in the plasma membrane due to a generation of ATP by the metabolism of glucose. As a consequence of the membrane depolarization, there is an opening of voltage-dependent Ca\(^{2+}\) channels. The increasing [Ca\(^{2+}\)]\(_i\) which follows is known to be a major trigger of insulin secretion. However, there are some recent indications that Ca\(^{2+}\)-independent pathways can mediate some of the effects of glucose in stimulating insulin secretion (Komatsu \textit{et al.} 1995).

Several alterations specific for the glucose signalling pathway in beta cells have been described in different diabetic rats such as a reduced ATP/ADP ratio, a low production of inositol 1,4,5-triphosphate (IP\(_3\)) and cAMP (Giroix \textit{et al.} 1993, Dachicourt \textit{et al.} 1996, 1997a,b, Morin \textit{et al.} 1996, 1997). Since ATP is one of the major metabolic signals associated with glucose, the reduced ATP/ADP ratio in beta cells would have a severe impact on the function of ATP-sensitive K\(^+\) channels in the plasma membrane and on the subsequent ionic events. Furthermore, it has been postulated that a diminished glucose-stimulated insulin secretion can be ascribed to abnormal [Ca\(^{2+}\)]\(_i\), handling of pancreatic islets. In this respect, the disappearance of glucose-induced [Ca\(^{2+}\)]\(_i\) oscillations in mouse beta cells treated with streptozotocin suggests that disturbances in [Ca\(^{2+}\)]\(_i\), may contribute to insulin secretory impairments in diabetes (Hellman \textit{et al.} 1990). Moreover, rat islets cultured at high glucose had a weaker increase in [Ca\(^{2+}\)]\(_i\), with a concomitant reduced insulin secretion in response to glucose as compared with freshly isolated pancreatic islets (Boschero \textit{et al.} 1990).

Another observed defect in [Ca\(^{2+}\)]\(_i\), handling that has been reported is the absence of the initial sequestration of Ca\(^{2+}\) by beta-cell sarco-endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) in diabetic db/db mouse islets (Roe \textit{et al.} 1994a). The lowering of [Ca\(^{2+}\)]\(_i\), represents an early action of glucose observed in normal mouse islets and depends on functional SERCA which is thought to play an important part in glucose regulation of insulin secretion.
of [Ca\(^{2+}\)]\(i\) responses to glucose in islets freshly isolated from diabetic rat models. Thus several atypical [Ca\(^{2+}\)]\(i\) responses to glucose could be expected to be present in islets issued from these two diabetic rat models.

In this context, our aim was to characterize the patterns of [Ca\(^{2+}\)]\(i\) responses to glucose in islets freshly isolated from GK and nSTZ diabetic rats. Our results show several major defects in [Ca\(^{2+}\)]\(i\), handling in both types of diabetic rat when acutely exposed to high glucose which are closely correlated to our previously reported alterations in their glucose-induced insulin secretion (Giroix et al. 1993).

Materials and Methods

Animals and islet isolation

The nSTZ-induced NIDDM (non-insulin-dependent diabetes mellitus) rat model was produced by a subcutaneous injection of streptozotocin (100 mg/kg) to male Wistar neonatal rats as previously described (Portha et al. 1974). All animals used in the experiments were adult male rats (12–13 weeks old). Plasma glucose (nmol/l) levels for normal Wistar, diabetic nSTZ and GK animals were (mean \(\pm\) s.e.m.) 6·6 \(\pm\) 0·2 (\(n=87\)) and 8·9 \(\pm\) 0·3 (\(n=77\)) (\(P<0·001\)) and 9·6 \(\pm\) 0·5 (\(n=51\)) (\(P<0·001\)) respectively. These diabetic rats were hyperglycaemic but no elevation of plasma triglyceride level could be observed (unpublished data). Islets of Langerhans were isolated from the pancreases by collagenase (Boehringer, Mannheim, Germany) digestion as described previously (Lacy & Kostianovsky 1967). The islets were hand picked under a stereomicroscope and loaded immediately with fura-2-acetoxymethyl ester or fura-2 (Molecular probes, Leiden, Netherlands).

Cytosolic [Ca\(^{2+}\)] measurements

Freshly isolated islets were loaded with 5 \(\mu\)M fura-2 during a 60-min incubation at 37 °C in a 10 mM HEPES-buffered medium (pH 7·4) containing (in mM): NaCl 124, KCl 5·9, CaCl\(_2\) 2·5, MgCl\(_2\) 1·2, glucose 2·8 and BSA (1 mg/ml). After loading, the islets were washed in the same buffer without BSA. Thereafter, they were allowed to attach on a polylysine-treated cover-glass placed at the bottom of an open perfusion chamber built for microscopic work (Liu et al. 1998). The chamber was made by placing a silicon rubber ring on top of the cover-glass which was held in place in a threaded chamber-mount by a thin stainless-steel ring. Cannulas feeding into the chamber were connected to a peristaltic pump and allowed a continuous superfusion of the islets with the above medium maintained at 37 °C at a flow rate of 1 ml/min. The effect of different agents could be tested at any time, when included in the medium. The chamber was placed on the stage of an inverted fluorescent microscope (Nikon, Diaphot, Champigny sur Marne, France) and maintained at 37 °C in a climate box. The microscope was equipped with a single quartz fibre illumination system and a ( \(\times\) 40) fluor oil immersion objective. A selected area of the islets was excited at 340 and 380 nm alternatively (every 2 s) and the fluorescence intensity emitted at 510 nm was measured by using a Photocas II microfluorimeter (Photon Technology International, Biotek Kontron, St Quentin Yvelines, France). Photodamage of the islet cells were minimized by using a minimal amount of illumination during analysis. Routinely, background fluorescence was recorded for both wavelengths in areas void of islets and the data was subtracted from the corresponding measurements of fura-2 loaded islets. The autofluorescence of islets was of the same value as that of background fluorescence estimated in the same measurement window. The measurements of successive 340/380 fluorescence ratios (\(R\)) reflect the cytosolic free calcium concentration which is abbreviated as follows: [Ca\(^{2+}\)]\(i\). The calculations of [Ca\(^{2+}\)]\(i\) were performed as previously described (Gylfe 1991) using fura-2 constants determined in small drops of ‘intracellular medium’ which was either depleted of Ca\(^{2+}\) (<1 nM) or contained 5 mM Ca\(^{2+}\).

Protocol and statistical analysis

For each experimental protocol, we studied one isolated islet with an average size of 70 \(\mu\)m picked from an islet pool obtained from two rat pancreases. This protocol was repeated 9–13 times for each type of experiment. Statistical analysis was performed using the ANOVA test combined with Fisher’s protected least significant difference (PLSD) test.

Results

Effect of 16·7 mM glucose on [Ca\(^{2+}\)]\(i\), of Wistar, GK and nSTZ rat islets

In normal Wistar islets, increasing the glucose concentration from 2·8 to 16·7 mM in the perfusate induced a lowering of [Ca\(^{2+}\)]\(i\), that was followed by a sharp rise reaching a maximal steady state after 10 min (Fig. 1A).
The corresponding magnitude of \([\text{Ca}^{2+}]_i\) increase over basal level was approximately 0.4 (Table 1) and slow and irregular oscillations were present. When 16.7 mM glucose was withdrawn and 2.8 mM glucose was added, there was a sharp drop in the \([\text{Ca}^{2+}]_i\). In contrast to this pattern obtained with normal Wistar islets, several alterations could be detected when monitoring the \([\text{Ca}^{2+}]_i\) responses of GK (Fig. 1B) and nSTZ (Fig. 1C) islets to a 16.7 mM glucose challenge. First, we could not observe in GK and nSTZ islets the initial drop of \([\text{Ca}^{2+}]_i\) induced by 16.7 mM glucose. Second, the time for a subsequent increase of \([\text{Ca}^{2+}]_i\) to 3% over basal levels was severely delayed in both GK and nSTZ islets as compared with Wistar islets (refer to the time of reaction in Table 1). Third, the rate of increase in \([\text{Ca}^{2+}]_i\) were respectively four- and twofold lower for GK and nSTZ islets as compared with that observed with Wistar islets (Table 1). Whereas the basal levels (90–107 nmol/l) of \([\text{Ca}^{2+}]_i\) were not significantly different in the three types of rat islets, the maximal increase over basal level observed with GK and nSTZ islets represented only 60% and 90% of the value obtained with Wistar islets. Finally, the sharp decrease in \([\text{Ca}^{2+}]_i\) after restoring 2.8 mM glucose observed in Wistar islets were not detected in GK and nSTZ islets. The above observed differences are unlikely to be due to islet size or composition since: islets of similar size (70 µm) were picked in all studies; the % of beta cells/total endocrine cells per islet of GK rats has been shown to be similar to that of Wistar rats (Giroix et al. 1999) while that of nSTZ rats was found slightly decreased (Giroix et al. 1992) and yet a difference in the magnitude of \([\text{Ca}^{2+}]_i\) increase induced by 16.7 mM glucose (Table 1) is only observed between control Wistar and GK groups.

**Sequestration of \([\text{Ca}^{2+}]_i\) in rat islets induced by glucose**

The first step of glucose effect, the initial sequestration of \([\text{Ca}^{2+}]_i\), which is absent in GK and nSTZ islets, was investigated using the \(K_{\text{ATP}}\) channel opener, diazoxide. This hyperpolarizing drug can be used to study effects of glucose which are independent of \(K_{\text{ATP}}\) channel and

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**Figure 1** Effects of 16.7 mM glucose on \([\text{Ca}^{2+}]_i\), as measured in a single pancreatic islet from either non-diabetic or diabetic rats. The \([\text{Ca}^{2+}]_i\) of freshly isolated islets from Wistar (A), GK (B) and nSTZ (C) rats was measured with fura-2 by using protocol as described in Materials and Methods. The fura-2 loaded islets were perfused with a buffer containing 2.8 mM glucose and the exposure to 16.7 mM glucose is indicated by the two broken lines. Each curve is representative of a typical \([\text{Ca}^{2+}]_i\) response to glucose obtained from results of 11–13 separate experiments performed in each group of animals. For each experimental protocol, we studied one isolated islet picked from an islet pool obtained from two rat pancreases. The changes in \([\text{Ca}^{2+}]_i\), are represented by the 340 nm/380 nm fluorescence ratio. Quantitative analysis of different parameters such as time of glucose to initiate either a decrease or increase in \([\text{Ca}^{2+}]_i\), over basal levels, rate of increase, and maximal \([\text{Ca}^{2+}]_i\), response are indicated in Table 1.
membrane potential (Trube et al. 1986 Gembal et al. 1993, Nadal et al. 1994). As shown in Fig. 2A, 250 μM diazoxide in presence of 2-8 mM glucose did not alter the basal [Ca^{2+}]i of Wistar islets, confirming that hyperpolarization alone is unable to lower [Ca^{2+}]i in islets. The initial drop of [Ca^{2+}]i in Wistar islets observed in response to the increased glucose concentration (16-7 mM) was not affected by 250 μM diazoxide (compare Figs 1A and 2A). This demonstrates that the sequestration of [Ca^{2+}]i occurs independently of Ca^{2+} influx in Wistar islets and this is absent in GK and nSTZ islets (Figs 2B and 2C). Furthermore, diazoxide inhibited the subsequent increase in [Ca^{2+}]i induced by 16-7 mM glucose in all three types of islets which were functional when verified by a pulse of 30 mM KCl.

The involvement of sarco-endoplasmic Ca^{2+}-ATPases (SERCA) in the initial lowering [Ca^{2+}]i induced by glucose was investigated with thapsigargin, a potent and selective inhibitor of SERCA (Thastrup et al. 1990). The data in Fig. 3A show that 1 μM thapsigargin abolished the initial reduction of [Ca^{2+}]i induced by 16-7 mM glucose without affecting the subsequent rise in [Ca^{2+}]i. This shows that the initial drop of [Ca^{2+}]i is due to a sequestration by thapsigargin-sensitive SERCA in Wistar islets and that the lack of the initial drop of [Ca^{2+}]i in islets observed in the two diabetic rats may implicate defective SERCA activity. Furthermore, thapsigargin revealed another defect in Ca^{2+} handling present in GK and nSTZ islets. Fast oscillations (3-5 ± 0-1 oscillations/min, n = 10) with high amplitudes were consistently observed following the rise in [Ca^{2+}]i in Wistar islets, as shown in Fig. 3A. This oscillatory effect on [Ca^{2+}]i, induced by thapsigargin could not be observed with GK (Fig. 3B) and nSTZ islets. However, in two out of ten islets tested from nSTZ islets, thapsigargin induced oscillations (1-3 and 1-5 oscillations/min), as shown in Fig. 3C.

**Discussion**

Two major dysfunctions in glucose homeostasis are known to be involved in the development of NIDDM: a reduced response to insulin in peripheral tissues and a reduced secretion of insulin from pancreatic beta cells. The GK and nSTZ rats, two recently developed animal models of NIDDM, have shared defects in the glucose-induced insulin secretion, such as a delayed onset, a reduction in the increment in insulin output and the maximal response (Giroix et al. 1993, Abdel-Halim et al. 1996). To further characterize the dysfunctions in insulin secretion, we have studied the cytosolic Ca^{2+} handling in islets freshly isolated from these two diabetic rats.

Common defects in the [Ca^{2+}]i responses in both GK and nSTZ islets to an acute increase in glucose concentration were observed and include: a delayed onset, a reduced rate of increment as well as a reduced maximal level as compared with Wistar islets. These data are coherent with the earlier mentioned defects in insulin secretion of these diabetic rats. Further, our finding of a delayed onset in [Ca^{2+}]i, increase in response to glucose substantiates earlier observations using cultured islets from GK rats (Zaitsev et al. 1997) and beta cells isolated from GK (Kato et al. 1996) as well as nSTZ islets (Tsuji et al. 1993). The reduced maximal [Ca^{2+}]i level in response to glucose which we observed in freshly isolated islets from GK rats was not seen in overnight cultured islets (Zaitsev

### Table 1

Quantitative analysis of glucose-induced [Ca^{2+}]i response in freshly isolated islets from Wistar, GK and nSTZ rats. The time of reaction corresponds to the time (s) it took to reach either a 3% decrease or increase of [Ca^{2+}]i as compared to basal values after the addition of 16-7 mM glucose. The basal values (90-107 nmol/l) for the different groups of animal were similar and 3% corresponded to approximately 3 mmol/l, a value previously used to consider changes of [Ca^{2+}]i from basal values (Gilon et al. 1994). The rate of increase in [Ca^{2+}]i, after addition of glucose is expressed as fluorescence ratio/s. The differences between maximal and basal fluorescence ratio values for each group of islets are also indicated. The number of experiments performed in each group of animals is indicated by n. For each experimental protocol, we studied one isolated islet picked from an islet pool obtained from two rat pancreases. Results are expressed as means ± S.E.M. and statistical analysis was performed as described in Materials and Methods.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Reduction</th>
<th>Increase</th>
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<tbody>
<tr>
<td>n</td>
<td>Time of reaction (s)</td>
<td>Time of reaction (s)</td>
</tr>
<tr>
<td>Wistar</td>
<td>11 46 ± 4</td>
<td>198 ± 20</td>
</tr>
<tr>
<td>GK</td>
<td>11 ND</td>
<td>321 ± 40**</td>
</tr>
<tr>
<td>nSTZ</td>
<td>13 ND</td>
<td>326 ± 40**</td>
</tr>
</tbody>
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*p < 0·05, **p < 0·01 and ***p < 0·001 as compared with Wistar rats. ND: not detected.

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[Ca^{2+}]_{i} of rat pancreatic islets measured in presence of diazoxide and glucose. The [Ca^{2+}]_{i} of freshly isolated islets from Wistar (A), GK (B) and nSTZ (C) rats was measured by fura-2 as indicated in Materials and Methods. The fura-2 loaded islets were perifused with a buffer containing 250 μM diazoxide in presence of either 2.8 mM glucose or 16.7 mM glucose as indicated by a broken line. At the end of each experiment, islets were exposed to 30 mM KCl in 2.8 mM glucose buffer without diazoxide as indicated by a solid horizontal line. Each curve is representative of results obtained from 9–12 separate experiments performed in each group of animals. For each experimental protocol, we studied one isolated islet picked from an islet pool obtained from two rat pancreases.

Figure 2

[Ca^{2+}]_{i} in isolated islets of diabetic rats

[Ca^{2+}]_{i} in isolated islets of diabetic rats

Figure 3

Effects of 16.7 mM glucose and 1 μM thapsigargin on [Ca^{2+}]_{i}, as measured in a single pancreatic islet from non-diabetic and diabetic rats. In these experiments, the fura-2 loaded islets obtained from Wistar (A), GK (B) and nSTZ (C) rats were perifused with a buffer containing 1 μM thapsigargin with either 2.8 mM glucose or 16.7 mM glucose as indicated. The measurement of [Ca^{2+}]_{i} was performed as described in Materials and Methods. At the end of each experiment, the islets were exposed to a 2.8 mM glucose buffer without thapsigargin. The curve is representative of results obtained from ten separate experiments performed on each group of animals. For each experimental protocol, we studied one isolated islet picked from an islet pool obtained from two rat pancreases.
et al. 1997). One likely explanation for this difference could be that the culturing conditions used, such as high glucose in culture medium and the duration of culture, can alter [Ca\textsuperscript{2+}] in islets obtained from GK and nSTZ rats since glucose infusion of rats can cause abnormal insulin response (de Souza et al. 2000) and cultured islets have both abnormal insulin and [Ca\textsuperscript{2+}] responses (Boschero et al. 1990).

A major difference identified in the Ca\textsuperscript{2+} handling between islets obtained from control and diabetic animals was that glucose initially lowered [Ca\textsuperscript{2+}], in islets obtained from Wistar rats while being ineffective in islets isolated from GK and nSTZ rats. That high glucose initially lowers [Ca\textsuperscript{2+}] in normal beta cells before a subsequent rise has been shown in cultured rat (Tsujii et al. 1993) and mouse beta cells (Gylfe 1988, Chow et al. 1995) and mouse islets (Gilon et al. 1994, Liu et al. 1998). This effect has not been clearly reported in islets issued from Wistar rats (Martin et al. 1995, Zaitsev et al. 1997) while in our hands it was consistently present. This effect has been attributed to active glucose-dependent sequestration of Ca\textsuperscript{2+} by sarco-endoplasmic reticulum Ca\textsuperscript{2+}-ATPases (SERCA) into the endoplasmic reticulum (Roe et al. 1994b). In favour of a sequestration is that the initial lowering of [Ca\textsuperscript{2+}], found in Wistar islets persisted in presence of diazoxide, a K\textsubscript{ATP} channel opener, used to show effects of glucose which are independent of the membrane potential (Trube et al. 1986, Gembal et al. 1993, Nadal et al. 1994). Thus, the lack of [Ca\textsuperscript{2+}] lowering in islets from both diabetic animals may reflect an impaired Ca\textsuperscript{2+} sequestration by SERCA and several observations sustain that this defect occurs in diabetic animals. For example, a lowered expression of SERCA which has been reported in islets isolated from nSTZ rats further upholds the impaired sequestration of [Ca\textsuperscript{2+}], oscillations in mouse islets (Longo et al. 1991, Bergsten 1995) while this remains unclear in rat islets (Martin et al. 1995). Further, it has been reported that thapsigargin by affecting SERCA can modify the frequency and amplitude of [Ca\textsuperscript{2+}], oscillations induced by glucose in mouse islets (Liu et al. 1998, Gilon et al. 1999). Thus, the absence of thapsigargin-induced fast oscillations in islets obtained from both GK and nSTZ rats further upholds the impairments of SERCA in these two NIDDM models.

In conclusion, by the comparative study of islets from GK and nSTZ rats, two well established models of NIDDM, we have revealed several common dysfunctions of [Ca\textsuperscript{2+}], handling in response to glucose, thus indicating a role in the poor insulin secretion observed in these animals. To what extent the impaired [Ca\textsuperscript{2+}], handling is related to abnormal sequestration of [Ca\textsuperscript{2+}], SERCA needs to be further clarified.

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