Glucose stimulation of pancreatic β cells induces oscillations of the membrane potential, cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)\(_i\)],) and insulin secretion. Each of these events depends on glucose metabolism. Both intrinsic oscillations of metabolism and repetitive activation of mitochondrial dehydrogenases by Ca\(^{2+}\) have been suggested to be decisive for this oscillatory behavior. Among these dehydrogenases, mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH), the key enzyme of the glycerol phosphate NADH shuttle, is activated by cytosolic [Ca\(^{2+}\)\(_i\)].

In this study, we compared different types of oscillations in β cells from wild-type and mGPDH\(^{-/-}\) mice. In clusters of 5–30 islet cells and in intact islets, 15 mM glucose induced an initial drop of [Ca\(^{2+}\)\(_i\)], followed by an increase in three phases: a marked initial rise, a partial decrease with rapid oscillations and eventually large and slow oscillations. These changes, in particular the frequency of the oscillations and the magnitude of the [Ca\(^{2+}\)\(_i\)] rise, were similar in wild-type and mGPDH\(^{-/-}\) mice. Glucose-induced electrical activity (oscillations of the membrane potential with bursts of action potentials) was not altered in mGPDH\(^{-/-}\) β cells. In single islets from either type of mouse, insulin secretion strictly followed the changes in [Ca\(^{2+}\)\(_i\)] during imposed oscillations induced by pulses of high K\(^+\) or glucose and during the biphasic elevation induced by sustained stimulation with glucose. An imposed and controlled rise of [Ca\(^{2+}\)\(_i\)] in β cells similarly increased NAD(P)H fluorescence in control and mGPDH\(^{-/-}\) islets. Inhibition of the malate-aspartate NADH shuttle with aminooxyacetate only had minor effects in control islets but abolished the electrical, [Ca\(^{2+}\)\(_i\)], and secretory responses in mGPDH\(^{-/-}\) islets. The results show that the two distinct NADH shuttles play an important but at least partially redundant role in glucose-induced insulin secretion.

The oscillatory behavior of β cells does not depend on the functioning of mGPDH and on metabolic oscillations that would be generated by cyclic activation of this enzyme by Ca\(^{2+}\).

Stimulation of insulin secretion by glucose involves a rise in the cytoplasmic concentration of Ca\(^{2+}\) ([Ca\(^{2+}\)\(_i\)],) in β cells (1, 2).

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3. The abbreviations used are: [Ca\(^{2+}\)\(_i\)], cytosolic free Ca\(^{2+}\) concentration; mGPDH, mitochondrial glycerol-3-phosphate dehydrogenase; AOA, aminooxyacetate; RT-PCR, reverse transcriptase-polymerase chain reaction.
membrane potential, $[\text{Ca}^{2+}]_i$, and insulin secretion in islet cell clusters or single islets from wild-type (mGPDH$^{+/+}$) and mGPDH$^{-/-}$ mice. The impact of an inhibition of the malate-aspartate shuttle by aminoxyacetic acid (AOA) (17, 29) was also evaluated in the two groups.

**EXPERIMENTAL PROCEDURES**

**Animals**—mGPDH expression and activity in islets were measured in Tokyo. To study the oscillatory behavior of the islets, female wild-type (mGPDH$^{+/+}$) and knockout (mGPDH$^{-/-}$) mice were transferred from Tokyo to Brussels. Their body weight, plasma glucose, and plasma insulin concentrations were $28.9 \pm 0.7$ g, $6.5 \pm 0.2$ mU/ml, and $1.15 \pm 0.14$ ng/ml, respectively, for wild-type mice, and $24.4 \pm 0.5$ g ($p < 0.05$), $6.2 \pm 0.2$ mU/ml (NS), and $0.94 \pm 0.11$ ng/ml (NS), respectively, for mGPDH$^{-/-}$ mice ($n = 11$ in each group).

**Analysis of mGPDH Expression by RT-PCR, Western Blot, and Enzymatic Activity**—Pancreatic islets were isolated (30) from mGPDH$^{+/+}$, $+/-$, and $-/-$ mice. Total RNA of islets was extracted using Isogen (Nippon Gene, Tokyo, Japan). The RNA (0.5 μg) was reverse-transcribed and PCR amplified in a single reaction tube catalyzed by a reverse transcriptase (Toyobo, Osaka, Japan). Two pairs of primers, 5'-CCCTGATTGACGAG-3' and 5'-ACGTGACCTTCCATCA-3', were used for amplification of mGPDH and glyceraldehyde-3-phosphate dehydrogenase gene transcripts, respectively. For detection of mGPDH protein by Western blot analysis, an anti-mGPDH antibody was raised against an oligopeptide (KTAEG-LDRVPIVPDVRSCGGL) corresponding to its carboxyl-terminal sequence, and was affinity-purified. Islets were homogenized in 0.23 μm mannnitol, 0.07 μl sucrose, and 5 mM potassium Hepes, pH 7.5, and electrophoresed in 10% SDS-polyacrylamide gel. Proteins were transferred to nitrocellulose membrane and the mGPDH signal was detected with an ECL system (Amersham Pharmacia Biotech). To measure mGPDH enzymatic activity in islet homogenates (300–500 islets per experiment) 2-p-iodo-3-nitro-5-phenyltetrazolium chloride (Sigma) was used as an electron acceptor, as described previously (31).

**Preparations Used to Study the Oscillatory Behavior of β Cells**—One wild-type and one mGPDH$^{-/-}$ mouse were usually killed on the same day. Their islets were isolated by collagenase digestion of the pancreas, followed by hand-picking (32). The medium was used in a bicarbonate-buffered solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM MgCl$_2$, 24 mM NaHCO$_3$, 10 mM glucose, and 1 mg/ml bovine serum albumin. It was gassed with O$_2$:CO$_2$ to maintain a pH of 7.4. The islets were then cultured for 1 or 2 days in RPMI 1640 medium containing 10 mM glucose, 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin. To obtain clusters of cells, some islets were incubated for 5 min in a Ca$^{2+}$-free medium. After brief centrifugation, this solution was replaced by culture medium and the clusters were disrupted by gentle pipetting through a siliconized glass pipette. The clusters were then cultured for 2 days on circular glass coverslips (33).

**Measurements of Insulin Secretion, [Ca$^{2+}$], and NAD(P)H**—The system has previously been described (23, 32), and the control medium was the same as that used for islet isolation. When the concentration of KCl was raised to 30 mM, that of NaCl was reduced to 94.8 mM. Cultured islets were loaded with fura-PE3 during 2 h of incubation at 37 °C in control medium containing 2 μM fura-PE3 acetoxymethyl ester. After loading, one islet was transferred into a 110-μl perfusion chamber with a bottom made of a glass coverslip and mounted on the stage of a microscope. The islet was held in place by gentle suction with a micropipette. The preparation was perfused at a flow rate of 1.8 μl/min and the medium was collected, in fractions of 30 s, just downstream of the islet. The temperature within the chamber was 37 °C. The [Ca$^{2+}$]$_i$ was measured by dual wavelength (340 and 380 nm) excitation spectrofluorimetry, using an CCD camera to capture images (510 ms) at 2.4-s intervals. From the ratio of the fluorescence at 340 and 380 nm, the concentration of [Ca$^{2+}$]$_i$ was calculated by comparison with a calibration curve (4). Insulin was measured, in duplicates, in 400-μl aliquots of the effluent fractions. The characteristics of the radioimmunoassay, using rat insulin as a standard, have previously been described in detail (32). The insulin content of the islets was determined after extraction in acid ethanol (34). It was similar in wild-type (112 ± 9 ng/islet) and mGPDH$^{-/-}$ mice (110 ± 7 ng/islet, $n = 22$). For the experiments in which only [Ca$^{2+}$]$_i$ was measured, 2–3 islets loaded with fura-PE3 were studied simultaneously in a larger chamber (~1 ml) perfused at a flow rate of 1.8 ml/min. When [Ca$^{2+}$]$_i$ was measured in clusters of islets cells, a coverslip with attached cells was first incubated in the medium containing fura-PE3/AM before being transferred into the perfusion chamber of which it formed the bottom. At the end of the experiment, the perfusion was stopped and the chamber filled with control solution containing 1 μM bisbenzimide. After 30 min, the preparation was excited at 365 nm and the number of cells in the studied cluster was measured by counting the fluorescent nuclei (at 510 nm) (35). The size of the studied clusters was similar for wild-type (14 ± 1 cell) and mGPDH$^{-/-}$ (16 ± 1 cell) mice.

**Presentation of Results**—All experiments have been performed with 1–3 islets from four to six different wild-type and mGPDH$^{-/-}$ mice. The results are illustrated by representative traces and/or presented as means (± S.E.). The statistical significance between means was assessed by unpaired or paired Student's t test as appropriate, and that of differences between percentages by Fisher's exact test. Differences were considered significant at $p < 0.05$.

**Fig. 1. Expression and activity of mGPDH in islets from mGPDH$^{+/+}$ (WT), mGPDH$^{+/-}$ (Hetero), and mGPDH$^{-/-}$ (Null) mice.**

A, RT-PCR analysis of mGPDH mRNA. Two hundred ng of total RNA extracted from islets was reverse-transcribed and amplified by 30 cycles of PCR (upper panel). As controls, transcripts of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene were amplified by 25 cycles of PCR (lower panel). B, Western blot analysis of mGPDH protein. Homogenates (100 μg of protein) prepared from islets were separated by SDS-polyacrylamide gel electrophoresis and blotted with anti-mGPDH antibody. C, enzymatic activity of mGPDH in islet homogenates (mean ± S.E.; four independent experiments with triplicate batches).
mGPDH expression and activity in islets—Reverse transcriptase-PCR analysis showed that mGPDH mRNA was not expressed in mGPDH\(^{-/-}\) islets (Fig. 1A, upper panel), whereas the glyceraldehyde-3-phosphate dehydrogenase gene transcript was amplified as effectively as that in wild-type islets (Fig. 1A, lower panel). The absence of mGPDH in mGPDH\(^{-/-}\) islets was confirmed by Western blot analysis with anti-mGPDH antibody (Fig. 1B), and by an assay of the enzymatic activity in islets homogenates (Fig. 1C). No mGPDH mRNA and protein was detected in muscle and liver tissues of mGPDH\(^{-/-}\) mice.\(^2\) The mGPDH mRNA was easily detectable in islets of heterozygous (mGPDH\(^{+/-}\)) mice, but the experimental conditions do not permit reliable quantification. The mGPDH protein and activity were decreased by about 40% in these heterozygous islets (Fig. 1).

Glucose-induced \([Ca^{2+}]_i\), changes in clusters of islet cells—In the presence of a non-stimulatory concentration of glucose (3 mM), \([Ca^{2+}]_i\), was low and stable (Fig. 2, upper panels). Raising the glucose concentration to 15 mM first induced a small drop in \([Ca^{2+}]_i\), that was rapidly followed by a marked increase in three phases: a long first phase, followed by a partial and progressive decrease with rapid oscillations, and eventually by large and slow oscillations. These changes were essentially similar in islet cell clusters from wild-type and mGPDH\(^{-/-}\) mice.

The characteristics of \([Ca^{2+}]_i\), oscillations occurring during steady state glucose stimulation are illustrated by the lower panels of Fig. 2. These oscillations often displayed a mixed pattern of small and fast transients superimposed on slower but larger ones (Fig. 2, B and E). In other cases only the slow oscillations were detected (Fig. 2, C and F). In no preparation continuously stimulated with 15 mM glucose were fast oscillations observed in the absence of slow ones. No difference could be identified in the appearance of the \([Ca^{2+}]_i\), oscillations in clusters from wild-type and mGPDH\(^{-/-}\) mice (Fig. 2). The frequency of the slow oscillations (0.25 ± 0.02 vs. 0.27 ± 0.02 per min, respectively) and the average \([Ca^{2+}]_i\), over 30 min of steady state stimulation with 15 mM glucose (283 ± 16 vs. 273 ± 16 nM, respectively) were also similar in the two types of islets.

Glucose- and Aminooxyacetate-induced \([Ca^{2+}]_i\), changes in intact islets—When islets were stimulated with 15 mM glucose, \([Ca^{2+}]_i\), initially decreased, then increased markedly and started to oscillate (Fig. 3). These oscillations were sometimes rapid (several per min) during the whole period of stimulation (not shown), became slower after a few minutes (Fig. 3, A and C), or were slow immediately after the first peak (not shown). Again these patterns were seen in both wild-type and mGPDH\(^{-/-}\) islets. The effect of AOA (used to inhibit the malate-aspartate shuttle) was, however, very different. Six to 7 min after addition of 5 mM AOA to the medium, \([Ca^{2+}]_i\), stopped to oscillate and returned to close to basal values in all mGPDH\(^{-/-}\) islets (Fig. 3, C and D). In wild-type islets, AOA only had a weak inhibitory effect, characterized by a decrease in the frequency of the oscillations. As shown in the inset of Fig. 3B, average \([Ca^{2+}]_i\), slowly but steadily increased with time in control islets continuously stimulated with 15 mM glucose alone. In contrast, \([Ca^{2+}]_i\), slightly decreased after addition of AOA and averaged 190 ± 6 nm between 35 and 40 min, which was significantly different \((p < 0.001)\) from the concentration measured in control islets without AOA (250 ± 5 nM) and in mGPDH\(^{-/-}\) islets treated with AOA (131 ± 6 nM).

Glucose-induced \([Ca^{2+}]_i\), oscillations were characterized further in experiments during which the islets were continuously stimulated with 12 mM glucose (Fig. 4). Two types of patterns were observed in wild-type islets: large and slow oscillations usually superimposed with smaller and faster ones (mixed pattern) (85%) (Fig. 4B) and fast oscillations only (15%) (Fig. 4A). The same patterns were observed in mGPDH\(^{-/-}\) islets.

\(^{2}\) K. Eto and T. Kadowaki, unpublished data.
Upon addition of AOA, 

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of 13 mGPDH respectively. (groups of islets treated with AOA (islets from wild type (mGPDH

duced by glucose and of their modifications by AOA in intact two groups of islets during perifusion with a medium containing 10 mM glucose. When the electrical activity was stable, the preparation was perfused with a medium containing 3 mM glucose for 10 min. The glucose concentration was then raised to 12 mM and 5 mM AOA was added 15 min later. The experiments were performed with islets cultured for 1 or 2 days. Records A and B were obtained in different mice. Records C and D show a continuous experiment without interruption. Complete experiments of this type were performed with 6 mGPDH−/− islets and 12 mGPDH+/+ islets. The mean electrical activity (calculated as % of time at plateau with spikes) is shown in panel E, where the dotted line corresponds to the mean electrical activity in 6 control islets stimulated with glucose alone until the end of the experiment.

The Oscillatory Behavior of Pancreatic Islets

Fig. 5. Effects of glucose (G) and AOA on the membrane potential of β cells within intact islets from wild-type (mGPDH+/+) and mGPDH−/− mice. β Cells were impaled with the microelectrode during perifusion with a medium containing 10 mM glucose. When the electrical activity was stable, the preparation was perfused with a medium containing 3 mM glucose for 10 min. The glucose concentration was then raised to 12 mM and 5 mM AOA was added 15 min later. The experiments were performed with islets cultured for 1 or 2 days. Records A and B were obtained in different mice. Records C and D show a continuous experiment without interruption. Complete experiments of this type were performed with 6 mGPDH−/− islets and 12 mGPDH+/+ islets. The mean electrical activity (calculated as % of time at plateau with spikes) is shown in panel E, where the dotted line corresponds to the mean electrical activity in 6 control islets stimulated with glucose alone until the end of the experiment.

potential then started to oscillate with bursts of spikes on top of each oscillation. During steady-state stimulation of wild-type islets with 10 or 12 mM glucose, the oscillations of the membrane potential were either regular and rapid (Fig. 5A), or displayed a mixed pattern (Fig. 5B) (50% of each pattern in 10 mM glucose, n = 20) (Fig. 5A). Both patterns were also seen in mGPDH−/− islets (46% regular and 54% mixed in 10 mM glucose, n = 13), and could sometimes be observed in the same cell (Fig. 5, C and D). The similarity of glucose-induced electrical activity in both types of islets is consistent with the unaltered properties of the K⁺/ATP channels in the β cell membrane of mGPDH−/− mice (30).

After about 5 min, AOA (5 mM) abolished the electrical activity induced by 12 mM glucose and repolarized the β cell membrane in mGPDH−/− islets (Fig. 5D). Fig. 5E is a quantification of the electrical activity induced by glucose (percentage of time at plateau potential with spikes) and of its inhibition by AOA. It first shows that glucose-induced electrical activity was quantitatively similar in the two types of islets. It then shows that AOA only had a small inhibitory effect in wild-type islets. After 15 min of AOA application, electrical activity was still present during 45 ± 5% of the time versus 67 ± 9% in control islets not treated with AOA (p < 0.05). This contrasts with the abrogation of electrical activity and the repolarization of β cells to a potential (−61 ± 3 mV) close to the resting potential in mGPDH−/− islets.

Correlations between \([\text{Ca}^{2+}]\), and Insulin Secretion Changes—In a first series of experiments, single wild-type and mGPDH−/− islets were perfused with a medium containing 15 mM glucose; diazoxide (100 μM) was also added to prevent glucose from depolarizing the membrane (38) and raising \([\text{Ca}^{2+}]\) (4). Oscillations of \([\text{Ca}^{2+}]\), were then imposed by repetitive 2-min depolarizations with 30 mM K⁺ (Fig. 6). Each of these triggered a peak of insulin secretion. The inactivation of mGPDH did not impair these responses.

In a second series of experiments, single islets were stimulated by 3 pulses (2.5 min) and a more sustained application (25 min) of 20 mM glucose in a control medium, without or with AOA. The upper panels of Fig. 7 illustrate individual responses,
and the middle panels show mean responses. In wild-type islets, each pulse of high glucose induced a large peak of [Ca$^{2+}$], accompanied by a peak of insulin secretion. The sustained stimulation caused an initial peak of [Ca$^{2+}$], followed by rapid oscillations, and a biphasic secretion of insulin (Fig. 7A). After the large first phase, the secretion rate dropped to lower values, which is typical for the mouse (37, 38). Fluctuations of secretion were usually found to follow the slow trends behind the fast [Ca$^{2+}$] transients, but no regular oscillations occurred. With collections every 30 s, the time resolution of the system is insufficient to monitor fast oscillations of secretion.

Several aspects of the responses of wild-type islets were altered when the experiments were performed in the presence of 5 mM AOA throughout (compare the middle and lower panels of Fig. 7, A and B). The rise in [Ca$^{2+}$], evoked by 20 mM glucose was clearly delayed, reaching a maximum only at the end of the 2.5-min glucose pulses, thus resulting in shorter [Ca$^{2+}$] oscillations, and a biphasic secretion of insulin (Fig. 7). Further glucose stimulation caused an initial peak of [Ca$^{2+}$], immediately upon glucose stimulation, which is best seen between 20 and 25 min (Fig. 7B, lower panel). During the long glucose application in the presence of AOA the elevation of [Ca$^{2+}$], displayed large oscillations (Fig. 7B, upper panel). Again, insulin secretion tightly followed the changes in [Ca$^{2+}$], with delayed and shorter peaks during glucose pulses, and collapsed oscillations synchronous with those of [Ca$^{2+}$], during sustained stimulation. Total insulin secretion was about 25% lower in the presence than absence of AOA, but this difference must be interpreted with caution because the experiments were performed with single islets whose individual insulin content could not always be determined.

When islets from mGPDH$^{-/-}$ mice were stimulated with 20 mM glucose alone, the [Ca$^{2+}$], and insulin secretion responses were essentially similar to those observed in wild-type islets (Fig. 7C). The situation was very different in the presence of 5 mM AOA (Fig. 7D). Raising the glucose concentration from 3 to 20 mM caused a drop in [Ca$^{2+}$], whereas the return to the low-glucose medium was followed by an increase in [Ca$^{2+}$]. This is most easily seen in the lower panel of Fig. 7D. During sustained stimulation with glucose, [Ca$^{2+}$], slightly increased after the initial fall, but remained much lower than in the absence of AOA. The upper panel of Fig. 7D illustrates the largest response in an mGPDH$^{-/-}$ islet treated with AOA. Under these conditions, insulin secretion was not stimulated at all.

**Effects of Glucose and [Ca$^{2+}$], on NAD(P)/H Autofluorescence**—For these experiments 100 μM diazoxide was added to the superfusion medium to hold [Ca$^{2+}$], at basal levels except in the presence of 30 mM KCl. Raising [Ca$^{2+}$], by high K$^+$ increased the NAD(P)H fluorescence at low and high glucose, but these stimulations were much smaller than that following the increase in glucose concentration from 3 to 15 mM (Fig. 8). The results were essentially similar in islets from wild-type and mGPDH$^{-/-}$ mice.

**DISCUSSION**

The mGPDH is the rate-limiting enzyme of the glyceraldehyde phosphate shuttle which, together with the malate-aspartate shuttle, permits reoxidation of cytosolic NADH by transferring reducing equivalents produced during glycolysis to the mitochondria. mGPDH is particularly abundant in pancreatic islets (16) and, in contrast to lactate dehydrogenase, much more so in β than non-β cells (12, 15). This peculiar biochemical organization (high mGPDH/lactate dehydrogenase ratio) is thought to be important for optimal coupling of glucose metabolism by the β cell and insulin secretion. Observations of a decreased activity of mGPDH in islets from type 2 diabetic patients and several animal models of the disease (11) have lent support to this concept, although recent studies have challenged the hypothesis (39, 40).

Mice with a targeted disruption of mGPDH have recently been generated and found to be grossly normal. In particular, they were not diabetic and their islets responded to glucose stimulation by similar increases in the ATP/ADP ratio and insulin secretion to those observed in control islets (21). The present study extends these findings in showing that the glucose-induced electrical activity and [Ca$^{2+}$], rise are quantitatively equivalent in wild-type and mGPDH$^{-/-}$ islets, and that the oscillatory characteristics of the electrical, ionic, and secretory events induced by glucose are similar in both types of islets. Since no mGPDH could be detected immunologically or enzymatically in mGPDH$^{-/-}$ islets it appears that the malate-aspartate shuttle can compensate for the absence of glyceraldehyde phosphate shuttle in all these biological functions.

AOA, an inhibitor of various aminotransferases, is widely used to block the malate-aspartate shuttle in islets and other tissues (17, 29, 41, 42). At the concentration of 5 mM, AOA inhibited glucose-induced insulin secretion by 50–60% in normal rat islets (17, 29). Smaller effects were observed in wild-type mouse islets. Glucose-induced electrical activity and [Ca$^{2+}$], rise were only attenuated and insulin secretion was slightly impaired and delayed. If the effects of AOA solely result from an inhibition of the malate-aspartate shuttle, which is uncertain owing to the drug action on several aminotransferases, our results would suggest that the malate-aspartate shuttle might exert functions that cannot be compensated for by the glyceraldehyde phosphate shuttle. However, the minor effects of AOA in control islets strikingly contrast with the abrogation by the drug of the electrical, [Ca$^{2+}$], and secretory responses to glucose in mGPDH$^{-/-}$ islets. It has been reported that the rise in the
ATP/ADP ratio that glucose produces in \( \beta \) cells (43) of normal islets (34) is markedly attenuated by AOA in mGPDH\(^{-/-}\) islets (21). This reflects a major alteration of glucose metabolism (21) and may explain why the \( \beta \) cell membrane was no longer depolarized and \( \text{Ca}^{2+} \) influx, manifested by the electrical activity, was no longer stimulated by glucose. At variance with a previous report (21), \([\text{Ca}^{2+}]_i\) remained low when mGPDH\(^{-/-}\) islets were challenged with glucose in the presence of AOA. There was thus no paradoxical dissociation between \([\text{Ca}^{2+}]_i\) and the suppression of insulin secretion. We, therefore, conclude that the NADH shuttles play an important role in glucose-induced insulin secretion and that the normal functioning of one of the two shuttles can largely compensate for an impairment of the other. This at least partial redundancy of the two NADH shuttles supports the importance of the system for the \( \beta \) cell functioning.

The major goal of this study was to evaluate whether mGPDH is involved in the oscillatory behavior of \( \beta \) cells. A number of events regularly oscillate in \( \beta \) cells during stimulation with a constant concentration of glucose. The origin of these oscillations is still incompletely understood (27), but a subtle interplay between metabolic and \([\text{Ca}^{2+}]_i\) changes may be involved. In this respect, the sensitivity of mGPDH to cytosolic \([\text{Ca}^{2+}]_i\) changes potentially confers a central position to the enzyme (28). The present results, however, do not support this hypothesis. Thus, no differences could be identified between glucose-induced oscillations of \( \beta \) cell membrane potential and \([\text{Ca}^{2+}]_i\) in wild-type and mGPDH\(^{-/-}\) \( \beta \) cells. The different phases of the changes (initial fall, large increase and oscillations) and the distinct patterns of the oscillations (regular fast or slow, and mixed fast and slow) were all observed in both types of islets. Their quantitative characteristics were also similar.

It has been proposed that a rise in \( \beta \) cell cytosolic \([\text{Ca}^{2+}]_i\) stimulates mitochondrial metabolism at the levels of mGPDH and intramitochondrial dehydrogenases (13, 14, 18, 19, 44). This mechanism is viewed as a feed-forward process promoting
ATP synthesis to sustain the secretory response. The present study, however, shows that repetitive stimulations with high glucose or high K⁺ induced pulses of insulin secretion from mGPDH⁻/⁻ islets, which did not differ from the control ones. No decrease in the response was observed, nor was there any tendency to a fall during the second phase of insulin secretion induced by sustained glucose stimulation. These results do not support the idea that mGPDH is a critical site where changes in cytosolic [Ca²⁺] play a regulatory role in glucose metabolism and subsequent functional events. Similar doubts have been raised by studies of the effects of Ca²⁺ on ATP production by islet mitochondria incubated with glycerol 3-phosphate (45). One should also keep in mind that the increase in NAD(P)H fluorescence, that follows glucose stimulation, is largely independent of an intracellular [Ca²⁺] rise (Fig. 7). In addition and even more importantly, the increase in NAD(P)H fluorescence brought about by Ca²⁺ in low or high glucose was not affected by the inactivation of mGPDH. [Ca²⁺] stimulation of glucose metabolism through activation of intramitochondrial dehydrogenases thus appears to be independent of the functioning of the glycerol phosphate shuttle and its activation by Ca²⁺.

In conclusion, NADH shuttles play an important role in the regulation of insulin secretion by glucose but seem to be at least partially redundant. Despite its [Ca²⁺] dependence and ability to display an oscillatory function in vitro, mGPDH is not the generator of metabolic signals that might in turn induce oscillatory biophysical and secretory responses in β cells.

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