Dihydroxyacetone-induced Oscillations in Cytoplasmic Free Ca\(^{2+}\) and the ATP/ADP Ratio in Pancreatic \(\beta\)-Cells at Substimulatory Glucose*

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Glucose stimulation of pancreatic \(\beta\)-cells causes oscillatory influx of Ca\(^{2+}\), leading to pulsatile insulin secretion. We have proposed that this is due to oscillations of glycolysis and the ATP/ADP ratio, which modulate the activity of ATP-sensitive K\(^+\) channels. We show here that dihydroxyacetone, a secretagogue that feeds into glycolysis below the putative oscillator phosphofructokinase, could cause a single initial peak in cytoplasmic free Ca\(^{2+}\) but did not by itself cause repeated oscillations in [Ca\(^{2+}\)]\(_i\) in mouse pancreatic \(\beta\)-cells. However, in the presence of a substimulatory concentration of glucose (4 mM), dihydroxyacetone induced [Ca\(^{2+}\)] oscillations. Furthermore, these oscillations correlated with oscillations in the ATP/ADP ratio, as seen previously with glucose stimulation. Insulin secretion in response to dihydroxyacetone was transient in the absence of glucose but was considerably enhanced and somewhat prolonged in the presence of a substimulatory concentration of glucose, in accordance with the enhanced [Ca\(^{2+}\)]\(_i\) response. These results are consistent with the hypothesized role of phosphofructokinase as the generator of the oscillations. Dihydroxyacetone may affect phosphofructokinase by raising the free concentration of fructose 1,6-bisphosphate to a critical level at which it activates the enzyme autocatalytically, thereby inducing the pulses of phosphofructokinase activity that cause the metabolic oscillations.

Glucose is the major secretagogue causing insulin secretion from the pancreatic \(\beta\)-cell. Its metabolism causes a rise in the ATP/ADP ratio, which closes ATP-sensitive K\(^+\) (K\(_{ATP}\)) channels, resulting in depolarization of the plasma membrane and influx of Ca\(^{2+}\) through voltage-gated Ca\(^{2+}\) channels. However, the rise in cytoplasmic free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) is not just static or monotonic. Glucose stimulation causes [Ca\(^{2+}\)] oscillations with a period of minutes, which correspond to oscillations in insulin secretion (1–3). Similar oscillations in insulin secretion are observed in vivo and are abnormal or diminished in type 2 diabetes (4–6); loss of oscillations may reduce the effectiveness of insulin (7, 8) and thus contribute to the development of the disease. In addition to this K\(_{ATP}\) channel-mediated action to raise [Ca\(^{2+}\)]\(_i\), there is also a K\(_{ATP}\) channel-independent stimulation of secretion by glucose (9, 10), which may involve the ATP/ADP ratio (9) or lipid signals (11). Because oscillations in insulin secretion can be seen under conditions in which [Ca\(^{2+}\)]\(_i\) is not changing, it has been proposed that the underlying oscillator is metabolic rather than ionic (12). In particular, we have proposed that the basis of these oscillations in the pancreatic \(\beta\)-cell is the spontaneous oscillations of glycolysis and the ATP/ADP ratio due to pulsed action of the muscle isoform of phosphofructokinase (PFK-M), which is present in \(\beta\)-cells (13). As demonstrated previously in muscle extracts, such oscillatory behavior can result from autocatalytic activation of PFK-M by its product, fructose 1,6-bisphosphate (Fru-1,6-P\(_2\)) (14, 15). The occurrence of glycolytic oscillations in the \(\beta\)-cell is supported by observations of similar oscillations in [Ca\(^{2+}\)]\(_i\), glucose 6-phosphate, and the ATP/ADP ratio (16, 17), as well as oscillatory release of lactate from perfused islets (18).

As a further test of this hypothesized role of glycolytic oscillations, we examined the actions of the trioses dihydroxyacetone (DHA) and glyceraldehyde (GA) that on phosphorylation feed into glycolysis downstream of the phosphofructokinase reaction. The trioses can also serve as secretagogues (19–21), though they have not been shown to cause oscillations in [Ca\(^{2+}\)]\(_i\) or insulin secretion. Of the two, GA is the more potent and the more widely studied. However, there is concern that it may not be a suitable glucose mimic in that it causes strong cellular acidification, most likely because of direct metabolism by glyceraldehyde-3-phosphate dehydrogenase and perhaps phosphoglycerate kinase to produce glyceric acid (22). We therefore focused on DHA, although some experiments were also performed with GA for comparison. Furthermore, we previously showed in the glycolyzing muscle extract system that the addition of DHA-P could trigger a pulse of PFK activity by

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The abbreviations used are: K\(_{ATP}\), ATP-sensitive K\(^+\) (channel); Fru-1,6-P\(_2\), fructose 1,6-bisphosphate; PFK, 6-phosphofructokinase (EC 2.7.1.11); PFK-M, phosphofructokinase, muscle isoform; GA, glyceraldehyde; DHA, dihydroxyacetone; DHA-P, dihydroxyacetone phosphate; BCECF, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; [Ca\(^{2+}\)]\(_i\), cytoplasmic free Ca\(^{2+}\) concentration; pH\(_i\), cytoplasmic pH.
raising the concentration of Fru-1,6-P₂ into the autocatalytic range (14). Here we show in mouse pancreatic β-cells that DHA by itself caused only a transient rise in [Ca²⁺]ᵢ, if any, but not oscillations. However, in the presence of a substimulatory concentration of glucose, the addition of DHA induced oscillations in [Ca²⁺]ᵢ, as well as corresponding oscillations in the ATP/ADP ratio. The glucose dependence suggests an effect of DHA to modulate glucose metabolism as in the muscle extract system and is consistent with the proposed role of PPK-M as the generator of the oscillations in the pancreatic β-cell.

**EXPERIMENTAL PROCEDURES**

**Isolation and Culture of Islet Cells—**Pancreatic islets were isolated from pancreas of 10–12 months old ob/ob mice (23), using a collagenase technique (24). Dispersed islet cells were prepared as described previously (25). The cell suspensions, consisting of more than 90% β-cells (26), were seeded onto glass coverslips. Cells were allowed to attach for about 1 h, and thereafter were submerged in RPMI 1640 medium (11 mM glucose) supplemented with 10% fetal calf serum, 100 μg/ml streptomycin, and 100 IU/ml penicillin. They were then cultured for 1–2 days. The solution used for isolation of islets and experiments contained 25 mM HEPES, pH 7.4, 125 mM NaCl, 5.9 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgCl₂, 1.2 mM bovine serum albumin, and the indicated concentrations of glucose.

**Fluorescence Measurements—**With the exception of the experiments for measuring ATP/ADP ratio, which needed larger numbers of cells in suspension (see below), all fluorescence measurements were made on single small-cell aggregates consisting of about 4–10 cells each, using a Spex Fluorolog spectrofluorometer coupled to a Zeiss Axiovert 35M microscope, with a Zeiss Fluor 40×/1.2 objective and a small open perfusion chamber mounted on a thermostatically controlled stage (27). For measurements of [Ca²⁺]ᵢ, cells were loaded with fura-2/AM (28), 1 μM, for 30 min at 37 °C. When rhodamine 123 fluorescence was used as a qualitative measure of mitochondrial membrane potential (29) simultaneously with fura-2 measurements of [Ca²⁺]ᵢ, this probe was added at 10 μg/ml during the last 5 min of the fura-2/AM incubation. When monitoring [Ca²⁺]ᵢ, and pHᵢ simultaneously, the cells were loaded with fura-2/AM and BCECF/AM concomitantly for 30 min, each at a concentration of 1 μM (27). Cells destined for NAD(P)H measurements were preincubated 30 min in the same medium without the probes. A fura-2/BCECF filter combination (diohroic mirror 505 nm and emission bandpass 515–565 nm) was used to monitor fura-2 fluorescence separately or in combination with BCECF or rhodamine 123 fluorescence.

Excitation wavelengths for fura-2 were 340/380 nm or just 380 in combination with the other probes. Both BCECF and rhodamine 123 were excited at 490 nm (29, 30). The excitation intensity levels were monitored with an excitation wavelength of 366 nm, a dichroic mirror 505 nm and emission bandpass 515–565 nm. However, in the presence of a substimulatory concentration of glucose, the addition of DHA induced oscillations in [Ca²⁺]ᵢ. That disappeared when DHA or glucose was omitted from the perfusion medium (Fig. 2) or when mannosepentose was added to prevent glucose metabolism (data not shown). The frequency of the oscillations was 0.23 ± 0.015 min⁻¹ (n = 11, three peaks or more in sequence, mean ± S.E.), similar to that seen with glucose stimulation (36). The oscillations were observed in 90% of the cell preparations in the simultaneous presence of 4 mM glucose and 10 mM DHA, and a sustained increase in [Ca²⁺]ᵢ was seen in the remaining preparations.

**RESULTS**

Effects of DHA and GA on [Ca²⁺]ᵢ—The ability of DHA and GA to initiate β-cell stimulation was first tested in the absence of glucose. As shown in Fig. 1, 11 mM glucose, 10 mM DHA, or 10 mM GA all produced a biphasic change in [Ca²⁺]ᵢ, with an initial small decrease followed immediately by a pronounced increase. Whereas GA always produced an increase in [Ca²⁺]ᵢ, DHA was less potent and in 30% of the experiments produced only a decrease or no change in [Ca²⁺]ᵢ (see also Fig. 2). Glucose (11 mM) caused an increase in [Ca²⁺]ᵢ, that was sustained for some minutes. GA produced a similar effect in 20% of the experiments and a transient effect in the rest, whereas DHA produced only a transient increase in [Ca²⁺]ᵢ, if any.

With regard to the ability of glucose to produce regular oscillations in [Ca²⁺]ᵢ, during prolonged β-cell stimulation, neither DHA nor GA alone was observed to have this effect. However, the combined presence of 4 mM glucose and 10 mM DHA produced regular oscillations in [Ca²⁺]ᵢ, that disappeared when DHA or glucose was omitted from the perfusion medium (Fig. 2) or when Mannosepentose was added to prevent glucose metabolism (data not shown). The frequency of the oscillations was 0.23 ± 0.015 min⁻¹ (n = 11, three peaks or more in sequence, mean ± S.E.), similar to that seen with glucose stimulation (36). The oscillations were observed in 90% of the cell preparations in the simultaneous presence of 4 mM glucose and 10 mM DHA, and a sustained increase in [Ca²⁺]ᵢ was seen in the remaining preparations.

GA (10 mM) could also produce oscillations in [Ca²⁺]ᵢ, in the presence of 4 mM glucose, but this was seen less consistently, in only about 25% of the preparations (data not shown), and a sustained increase was observed in the remaining preparations. Because neither DHA nor GA

**Fig. 1. Initial effects of glucose, GA, and DHA on [Ca²⁺]ᵢ in islet cell aggregates.** Fura-2-loaded islet cell aggregates perfused in the absence of glucose were stimulated with 11 mM glucose, 10 mM GA, or 10 mM DHA during the time indicated by the black horizontal bar. The data are displayed as fura-2 340/380 ratio values and have been normalized to the values at the beginning of each experiment. The traces presented are representatives of 18 of 18 experiments with glucose, 14 of 14 experiments with GA, and 16 of 23 experiments with DHA. In 7 of 23 experiments with DHA, no increase or a slight decrease in the fura-2 340/380 ratio was observed (see Fig. 2B).
by themselves evoked oscillations in [Ca\(^{2+}\)], but perhaps only a single rise in [Ca\(^{2+}\)], these findings may suggest that the actual mechanism responsible for the slow glucose-induced oscillations in [Ca\(^{2+}\)], is located upstream of the triose level, most likely at phosphofructokinase, which is a known generator of oscillations of glycolysis and the ATP/ADP ratio (12).

In a separate set of experiments, [Ca\(^{2+}\)], measurements were performed using CCD imaging to monitor individual cells and also to test a higher concentration of 20 mM DHA. Cells in the absence of glucose were first stimulated with DHA (20 mM) alone for 15 min, returned to a base line at 4 mM glucose and then stimulated with 10 mM DHA plus 4 mM glucose for 15 min, again returned to 4 mM glucose alone, and finally stimulated with 8 mM glucose. The initial peak was followed by a slightly elevated concentration if anything may have decreased somewhat, to 28 μM at 10 min and about the same at 13 min; this was similar to the initial 20 mM DHA stimulation, the glucose concentration was dropped from 4 to 0 mM for 60 s. Of 139 cells recorded (in 40 cell clusters from 5 mice), 48 showed oscillations in response to DHA in the absence of 4 mM glucose, but only 1 cell showed oscillations in the absence of glucose.

DHA-induced Oscillations in the ATP/ADP Ratio—When suspensions of mouse islet cells were stimulated with 10 mM DHA in the presence of 4 mM glucose, the ATP/ADP ratio increased (Fig. 4), although to a considerably smaller extent than seen previously with high glucose (16). Furthermore, although of lower amplitude than with glucose, there were oscillations in the ATP/ADP ratio corresponding to the [Ca\(^{2+}\)], oscillations.

We attempted to measure the accumulation of Fru-1,6-P\(_2\) and triose-P using enzymatic cycling assays (34). However, this was initially found not to be practical because of interference from the high level of DHA from the incubation medium. DHA was a poor, but nevertheless overwhelming, substrate for glyceraldehyde-3-phosphate dehydrogenase in comparison with the cellular levels of DHAP and Fru-1,6-P\(_2\) in these samples. We subsequently tried batch incubations of larger amounts of material in 4 or 8 mM glucose for 10 min or 4 mM glucose plus 10 mM DHA for 10 or 13 min, utilizing anion exchange chromatography to remove the interfering DHA. At 4 mM glucose, the Fru-1,6-P\(_2\) level was 0.55 ± 0.11 pmol/islet (mean ± S.E., n = 3), or 50 μM, calculated assuming 1.1 pl/cell and 10,000 cells in an ocell of mouse islet (37). With DHA, the Fru-1,6-P\(_2\) concentration if anything may have decreased somewhat, to 28 ± 8 μM at 10 min and about the same at 13 min; this was similar to the concentration in the presence of 8 mM glucose, 32 ± 12 μM. These Fru-1,6-P\(_2\) concentrations would probably be nearly saturating for activation of PFK-M (13, 38). On the other hand, the triose-P concentration of 41 ± 5 μM at 4 mM glucose most likely indicates a much lower free concentration of Fru-1,6-P\(_2\) of 0.7 ± 0.2 μM, based on equilibration of the triose-phosphate isomerase and aldolase reactions (see “Discussion”). Calculated free concentrations of Fru-1,6-P\(_2\) varied considerably after 10 or 13 min of incubation with DHA, ranging from 26 to 364% of the values in 4 mM glucose alone.

Effects of DHA ± Substimulatory Glucose on Insulin Secretion—The addition of 10 mM DHA to perifused islets in the absence of glucose caused a small transient release of insulin (Fig. 5). However, the addition of DHA in the presence of 4 mM glucose, which in itself was not stimulatory, caused substantially more insulin release. Most of this was in the initial peak, which was about five times greater than in the absence of glucose. The initial peak was followed by a slightly elevated
plateau. When high (11 mM) glucose (without DHA) was added after DHA at 0 mM glucose, there was a large initial peak and an elevated plateau (Fig. 5) similar to the behavior seen in other experiments without an intervening interval of DHA (data not shown). Interestingly, when high glucose was added after DHA at 4 mM glucose, the initial peak response was lost, although a similar elevated plateau was maintained.

**Effects of DHA and GA on Mitochondrial Membrane Potential, NAD(P)H Fluorescence and pHi**

To examine the effects of the trioses on mitochondrial energetics, rhodamine 123 and fura-2 were used simultaneously to monitor mitochondrial membrane potential and [Ca^{2+}]_{i}. A decrease in rhodamine 123 fluorescence, indicative of mitochondrial hyperpolarization (29), preceded the glucose-induced increase in [Ca^{2+}]_{i} by 51 ± 9 s (mean ± S.E., n = 7) (Fig. 6A). DHA and GA, in the absence of glucose, also caused mitochondrial hyperpolarization (Fig. 6, B and C). In the case of GA the time delay between mitochondrial hyperpolarization and the increase in [Ca^{2+}]_{i} was 52 ± 7 s.

**FIG. 4. Coordinate oscillations in [Ca^{2+}]_{i} and the ATP/ADP ratio induced by DHA in the presence of 4 mM glucose in a suspension of mouse β-cells.** [Ca^{2+}]_{i} in a suspension of cells was monitored continuously with fura-2 (upper panel). Glucose (4 mM) was present throughout, and DHA (10 mM) was added as indicated by the horizontal bar. Samples were taken at the times indicated in the lower panel and assayed for ATP and ADP. Data are means ± S.E. of triplicate measurements in one experiment, except that the penultimate point was a single measurement. The experiment was repeated four times with similar results.

**FIG. 5. Effect of DHA with and without glucose on insulin secretion from perfused islet cells.** Cell aggregates were perfused without glucose (open symbols) or with 4 mM glucose (filled symbols), and DHA (10 mM) was added for the time period shown by the bar; subsequently DHA was omitted, and the glucose concentration was raised to 11 mM. Insulin release is expressed relative to the mean insulin release during the first 20 min. Basal release did not differ between 0 and 4 mM glucose. Data are means ± S.E. of four experiments.

**FIG. 6. Effects of glucose (G) (panel A), DHA (panel B), and GA (panel C) on [Ca^{2+}]_{i} and mitochondrial membrane potential in cell aggregates, measured simultaneously with fura-2 (380 nm) and rhodamine 123 (490 nm).** The substances were introduced as indicated by the horizontal bars: A, 11 mM glucose; B, 10 mM DHA followed by 4 mM glucose; C, 10 mM GA. No glucose was present initially. The decrease of fura-2 fluorescence at 380 nm indicates a rise in [Ca^{2+}]_{i}. The decrease in rhodamine 490 nm fluorescence indicates mitochondrial hyperpolarization. The dotted line in panel B (490 nm) was added to clarify the small drop in rhodamine 123 fluorescence upon subsequent introduction of 4 mM glucose. The traces are representatives of 6 experiments (A), 4 of 5 experiments performed (B), and 4 experiments (C) performed on different cell preparations. In one of five experiments with experimental protocol B, [Ca^{2+}]_{i} increased shortly after the introduction of DHA. The time courses for the decreases in fura-2 and rhodamine 123 fluorescence in this experiment were similar to the traces shown in A and C.
Dihydroxyacetone and Ca\(^{2+}\) Oscillations

**DISCUSSION**

The studies presented here show that DHA, which feeds into glycolysis downstream of the phosphofructokinase step, did not by itself cause oscillations in [Ca\(^{2+}\)] in mouse \(\beta\)-cells. However, it could induce [Ca\(^{2+}\)], oscillations when added in the presence of a substimulatory concentration of glucose. These [Ca\(^{2+}\)], oscillations correlated with oscillations in the ATP/ADP ratio (Fig. 4), as shown previously for glucose stimulation (16), suggesting a similar metabolic basis. A likely explanation for this action of DHA is suggested by earlier experiments with the glycolyzing muscle extract system. We demonstrated that oscillations in the muscle extract were caused by repeated bursts of PFK-M activity and that a burst began when Fru-1,6-P\(_2\) accumulated to a critical level of about 1 \(\mu\)M and began to activate the enzyme autocatalytically (14). Furthermore, because DHA-P is in equilibrium with Fru-1,6-P\(_2\) through the triose-phosphate isomerase and aldolase reactions, it was found that the addition of DHA-P could trigger an early oscillation if the amount added was sufficient to raise the Fru-1,6-P\(_2\) concentration to that critical level (14). In the experiments with \(\beta\)-cells presented here, addition of DHA may have had a similar effect after phosphorylation to DHA-P, by raising the concentration of Fru-1,6-P\(_2\) to the critical level to trigger bursts of PFK activity and thus oscillations of glycolysis and the ATP/ADP ratio. This, of course, would occur only in the presence of some glucose to provide substrate for PFK.
We were unable to demonstrate a rise in Fru-1,6-P_2 with DHA added in the presence of 4 mM glucose. Furthermore, the assayed concentrations of 30–50 μM Fru-1,6-P_2 were well above the level necessary to begin to activate PFK-M. However, this does not negate the above explanation, because of several practical considerations. First, it is the free concentration of Fru-1,6-P_2 that would be important in the regulation of PFK-M, but it is the total concentration that is assayed. In other tissues studied previously, up to 99% of the Fru-1,6-P_2 appeared to be bound (14, 38). Similarly, in the islet cells, the free Fru-1,6-P_2 concentration that would be in equilibrium with the assayed triose-P levels may be calculated to be about 0.7 μM in the presence of 4 mM glucose, using values of 28 and 90 μM for the triose-phosphate isomerase and aldolase equilibrium constants, respectively (40). A second complicating factor is that the level of a metabolite varies in an oscillating system, sometimes upward and sometimes downward. This may be part of the reason that the level of Fru-1,6-P_2 appeared to decrease sometimes in these samples taken 10 or 13 min after stimulation with DHA or glucose. Furthermore, as shown in the muscle extract system, the average Fru-1,6-P_2 concentration may be much larger than the trigger level of Fru-1,6-P_2 needed to start autocatalytic activation (14).

DHA added in the presence of substimulatory (4 mM) glucose caused substantially more insulin secretion than when added in the absence of glucose (Fig. 5). The oscillations in [Ca^{2+}], induced by DHA in the presence of substimulatory glucose (Figs. 2 and 3) amounted to a prolonged increase in average [Ca^{2+}], similar to that seen with high glucose, and this probably accounted for the increase in insulin secretion under these conditions. Nevertheless, DHA was still less effective than high glucose, which suggests that the magnitude of the secretory response is in part dependent on other factors normally produced by high glucose, such as lipid signaling molecules (11). Furthermore, the addition of DHA in the presence of 4 mM glucose appeared to prevent the initial high peak of insulin release on subsequent stimulation with high glucose, which perhaps indicates that DHA may be less effective than glucose in replenishing the releasable pool of granules.

There have been few previous studies of DHA stimulation of secretion, with somewhat differing results. Zawalich et al. (20, 21) reported maximal stimulation of second phase secretion in perfused rat islets at 20 mM DHA, with half-maximal stimulation at 5 mM and near maximal by 10 mM, similar to the results of Hellman et al. (19) for batch incubated ob/ob mouse islets. Some stimulation by DHA of human islets (41) and clonal β-cells (HIT) (42) has also been observed. On the other hand, several groups have reported no stimulation by DHA of batch-incubated rat islets in the absence of glucose (43–45). However, one of these studies (44) reported a doubling of basal secretion when DHA (5–10 mM) was added together with low (2.8 mM) glucose. These latter reports seem more similar to our observations. As noted by Zawalich (21), differences in the metabolic state of the animal and in the islet preparation and study protocols may be responsible for some of these differing results.

Glucose, DHA, and GA could all cause marked effects on mitochondrial metabolism, as indicated by the measurements of mitochondrial membrane potential, before the appearance of an increase in [Ca^{2+}]. This has previously been inferred for glucose from parallel measurements of [Ca^{2+}], and rhodamine 123 fluorescence (29) and was demonstrated recently with simultaneous measurements (46), but it was not shown previously for DHA and GA. However, in contrast to glucose, neither DHA nor GA produced a detectable increase in NAD(P)H fluorescence (29). A possible explanation in the case of GA comes from the suggestion that GA may be oxidized directly by glyceraldehyde-3-phosphate dehydrogenase to produce cytosolic NADH, which is reoxidized via the glycerol phosphate shuttle, feeding into the electron transport chain downstream from mitochondrial NADH (22). As noted above, NAD(P)H fluorescence measurements largely reflect mitochondrial NADH.

The presently observed timing and sequence of changes in pH and [Ca^{2+}], upon the addition of glucose are similar to previous reports in which both parameters were measured simultaneously (27) or in parallel (47). The glucose-induced changes in pH may affect various components of the stimulus-secretion coupling mechanism (27), but they can also serve as indicators of underlying metabolic or ionic fluxes. We showed previously that the initial rise in pH could be blocked by the mitochondrial pyruvate transport inhibitor 3-hydroxycynocinnamate, without affecting the subsequent Ca^{2+} signal (27). This indicated that the alkalinization was most likely due to enhanced mitochondrial metabolism of pyruvate, probably in response to the ATP usage in the initial glucose phosphorylation(s). The alkalinization seen here with DHA may therefore be a reflection of its phosphorylation. Glucose and DHA caused a subsequent decrease in pH, perhaps due to glycolytic acid (pyruvate) production or activation of H^{+}/Ca^{2+} exchange by the increase in [Ca^{2+}] (47). In contrast to the actions of glucose and DHA, GA caused only a strong and early acidification. It is clear from the present data that the mechanism behind the decrease in pH induced by GA is different from the one caused by glucose and DHA and not secondary to Ca^{2+} influx because it preceded the increase in [Ca^{2+}]. The lowering effect of GA on pH may be due to the accumulation of acidic metabolites such as glycerate, a property that could explain its cytotoxic effects in pancreatic islets after overnight culture (22). For these reasons and other possible interfering effects of the acidification (see below), GA may be less suitable for metabolic studies of fuel stimulus-secretion coupling. The acidification by GA and alkalinization by DHA have been reported previously (30, 48–50), but here the changes in pH, and [Ca^{2+}], have been measured simultaneously for the trioses.

In these studies GA was much less consistent than DHA in inducing oscillations in [Ca^{2+}], in the presence of glucose. This may be due to the GA-induced drop in pH, which may have effects such as closure of K_ATP channels (48, 51, 52), inhibition of gap junctional conductance (53) (which may be important for electrical coupling to promote oscillatory behavior), and inhibition of PFK-M, the putative generator of the oscillations, because the enzyme has been shown to be extremely pH-sensitive (53, 54). Indeed, we previously showed in the glycolyzing muscle extract system that differences in the initial pH of 0.1 unit or less could greatly affect the time of initiation and the period of the oscillations (55). On the other hand, since the present studies were done, Lenzen et al. (56) have reported the induction of [Ca^{2+}], oscillations in mouse islets by GA in the presence but not in the absence of 5 mM glucose. The results of these studies are consistent with our hypothesis that PFK-M is the generator of the oscillations.

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