Fructose-induced increase in intracellular free Mg^{2+} ion concentration in rat hepatocytes: relation with the enzymes of glycogen metabolism

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In rat hepatocytes subjected to a fructose load, ATP content decreased from 3.8 to 2.6 μmol/g of cells. Under these conditions, the intracellular free Mg^{2+} ion concentration, as measured with mag-fura 2, increased from 0.25 to 0.43 μmol/g of cells and 0.35 μmol of Mg^{2+} ions were released per g of cells in the extracellular medium. Therefore the increase in the intracellular free Mg^{2+} ion concentration was less than expected from the decrease in ATP, indicating that approx. 80 % of the Mg^{2+} ions released from $MgATP^{2-}$ were buffered inside the cells. When this buffer capacity was challenged with an extra Mg^{2+} ion load by blocking the fructose-induced Mg^{2+} efflux, again approx. 80 % of the extra Mg^{2+} ion load was buffered. The remaining 20 % appearing as free Mg^{2+} ions in fructose-treated hepatocytes could act as second messenger for enzymes having a K_m for Mg^{2+} in the millimolar range. Fructose activated glycogen synthase and glycogen phosphorylase, although both the time course and the dose-dependence of activation were different. This was reflected in a stimulation of glycogen synthesis with concentrations of fructose below 5 mM. Indeed, activation of glycogen synthase reached a maximum at 30 min of incubation and was observed with small (5 mM or less) concentrations of fructose, whereas the activation of glycogen phosphorylase was almost immediate (within 5 min) and maximal with large doses of fructose. The fructose-induced activation of glycogen phosphorylase, but not that of glycogen synthase, could be related to an increase in free Mg^{2+} ion concentration.

INTRODUCTION

The intracellular total concentration of Mg^{2+} ions in the liver (approx. 10 μmol/g wet weight [1]) greatly exceeds the intracellular free concentration (approx. 0.5 mM [2,3]), indicating that this bivalent cation is mainly sequestered. The Mg^{2+} ion content of hepatocytes depends on the efflux of Mg^{2+} ions by Na^+/Mg^{2+} exchange [4], and on the influx of Mg^{2+} by Na^+, Mg^{2+}/anion co-transport [5]. Mg^{2+} movements across the plasma membrane and from intracellular stores are hormonally regulated [6–12]. Fluctuations in intracellular free Mg^{2+} ion concentration ($[Mg^{2+}]_i$) can also result from the storage or release of Mg^{2+} ions by intracellular ionic ligands. The most abundant intracellular Mg^{2+} buffer is probably ATP, which exists within the cell as $MgATP^{2-}$ [13]. Depletion of cellular ATP by, for example, anoxia increases $[Mg^{2+}]_i$ in various cell types [13–17], including hepatocytes [14,15]. This was measured by ^{31}P-NMR [14] or with the fluorescent probe mag-fura 2 [15–17].

A fructose load also decreases ATP concentration in the liver or in hepatocytes. Indeed, fructose is rapidly phosphorylated to fructose 1-phosphate, which accumulates and acts as a trap for P_i [18,19]. In contrast with anoxia, a fructose load induces a loss of total adenine nucleotides and ATP resynthesis does not follow because of P_i depletion [18,19]. Under these conditions, an increase in $[Mg^{2+}]_i$ is expected. We therefore studied the effect of fructose on $[Mg^{2+}]_i$, as measured with mag-fura 2, in isolated hepatocytes. Fructose affects the metabolism not only of adenine nucleotides but also of glycogen [20]. Because fructose is known to activate glycogen synthase [20], we also considered the possibility that an increase in $[Mg^{2+}]_i$ could participate in glycogen synthase activation by stimulating protein phosphatase activity.

EXPERIMENTAL

Materials

Mag-fura 2 acetoxymethyl ester (AM), fura 2/AM and Pluronic F127 were obtained from Calbiochem. All other materials and biochemicals were from Sigma or Boehringer.

Measurement of intracellular free Mg^{2+} and Ca^{2+} ion concentrations with single excitation wavelength

Hepatocytes [21] were prepared from overnight-fasted male Wistar rats and resuspended at 10–20 mg wet weight/ml in Krebs–Henseleit bicarbonate buffer [22] in equilibrium with a gas phase containing O_2/CO_2 (19:1), and supplemented with 10 mM glucose (buffer A) and 1.5 % BSA. $MgSO_4$ or NaCl was replaced in the Krebs–Henseleit buffer by 2.3 mM NaCl or 118 mM choline chloride to obtain a Mg^{2+}-free or choline buffer respectively. To load the cells with the fluorescent probe, hepatocytes were washed with buffer A and incubated (10–20 mg wet weight/ml) with 5 μM mag-fura 2/AM for 20 min at 37 °C [2] or with 5 μM fura 2/AM supplemented with 0.025 % Pluronic F127 for 30 min at 37 °C [23]. The cells were then washed with buffer A or with Mg^{2+}-free or choline buffer and transferred to a 2 ml quartz cuvette in a Perkin-Elmer LS-5 fluorimeter. The cuvettes were thermostatically controlled at 37 °C and the hepatocyte suspension was continuously stirred and oxygenated. $[Mg^{2+}]_i$ was measured in mag-fura 2-loaded cells with the excitation wavelength at 370 nm and the emission wavelength at 510 nm. The measurement of intracellular free Ca^{2+} ion concentration ($[Ca^{2+}]_i$) was performed in fura 2-loaded hepatocytes with excitation and emission wavelengths at 380 and 505 nm

Abbreviations used: AM, acetoxymethyl ester; $[Ca^{2+}]_i$, intracellular free Ca^{2+} ion concentration; $[Mg^{2+}]_i$, intracellular free Mg^{2+} ion concentration.

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respectively [24,25]. An increase in $[Mg^{2+}]_i$ or $[Ca^{2+}]_i$ is reflected by a decrease in fluorescence. Calibration of mag-fura 2 and fura 2 signals was made after the addition of digitonin (final concentration 10 μM) to measure the fluorescence, F_{max}, of the probe in the presence of an excess of Mg^{2+} (1.15 mM) or Ca^{2+} (2.5 mM) ions, followed by the addition of EDTA (mag-fura 2) or EGTA (fura 2) at a final concentration of 25 mM to determine the fluorescence, F_{min}, in the absence of Mg^{2+} or Ca^{2+}. $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ were calculated [24,26] using 1.5 mM as K_D for the Mg/mag-fura 2 complex [2] and 224 nM as K_D for the Ca/fura 2 complex [24]. Autofluorescence represented less than 5 % of the fluorescence obtained with mag-fura 2-loaded cells and none of the effectors used interfered with the autofluorescence at the wavelengths used to monitor $[Ca^{2+}]_i$.

$[Mg^{2+}]_i$ measurement with dual excitation wavelengths

Hepatocytes were prepared and incubated as described above with 5 μM mag-fura 2/AM for 20 min. The cells were then washed with buffer A and placed into an oxygenated and thermostatically controlled (37 °C) chamber mounted on an inverted microscope with a Fluor × 100 oil-immersion objective. Fluorescence was monitored within a single hepatocyte over the course of the study. Fluorescence was recorded with a dual-excitation-wavelength spectrofluorimeter with excitation at 335 and 370 nm and emission at 510 nm. All additions were made by perifusing the cells with buffer A supplemented with the indicated effectors. Calibration of the signal was not possible because the addition of digitonin leads to the release and loss of mag-fura 2 in the perifusing buffer [2].

Measurement of Mg^{2+} ion efflux

Hepatocytes were incubated (50–70 mg wet weight/ml) for 15 min at 37 °C in Mg^{2+}-free buffer or in Mg^{2+}-free choline buffer made as described above, and supplemented with 10 mM glucose. The indicated effectors were then added and the cells were incubated for a further 5 and 30 min. At the end of the incubation, samples were withdrawn and centrifuged in Microfuge tubes (Microfuge, full speed for 5 s). Proteins present in the supernatant were precipitated in the presence of 10 % (w/v) trichloroacetic acid. The Mg^{2+} ion content of the supernatant was measured by atomic absorbance flame spectrophotometry. The quantity of Mg^{2+} ions (in μmol) present in the supernatants were expressed per g of cells. The basal loss of Mg^{2+} ions amounted to 2 μmol/g of cells after 5 and 30 min of incubation and was similar to that observed by others [7].

Measurements of metabolites and enzyme activities

Hepatocytes were incubated (50 mg wet weight/ml) at 37 °C in Krebs–Henseleit bicarbonate buffer [22] containing the indicated additions. At the indicated times, aliquots were withdrawn for metabolite and enzyme activity measurements. ATP was measured in neutralized perchlorate extracts by a coupled enzyme assay [27]. Glycogen content was measured in alkaline extracts [28] and is expressed as glucose equivalents. The active forms of glycogen synthase [29] and glycogen phosphorylase [30] were measured at 25 °C as described. Enzyme activity is expressed as units, i.e. μmol of substrate consumed per min under the assay conditions. The values shown are the means ± S.E.M. for the given number of different preparations of hepatocytes. The statistical significance of differences was calculated by Student's t test (paired data).

RESULTS

Effect of fructose on $[Mg^{2+}]_i$ and ATP content

Figure 1 shows the time course of the effect of fructose on the fluorescence of mag-fura 2-loaded hepatocytes. When a stable signal had been obtained for 5 min under control conditions, addition of fructose at a final concentration of 10 mM decreased the signal by 10 %, i.e. about doubled $[Mg^{2+}]_i$, within 3 min. Calibration of the signal gave a basal $[Mg^{2+}]_i$ of 0.50 ± 0.11 mM ($n = 3$). This is similar to the 0.40–0.50 mM reported for rat hepatocytes [2,3] or perfused rat livers [31]. The time course of the reciprocal changes in ATP content and $[Mg^{2+}]_i$ after a fructose load is shown in Figure 2. In the presence of 10 mM fructose, $[Mg^{2+}]_i$ increased from 0.50 ± 0.11 mM to 0.86 ± 0.16 mM after 3–5 min (Figure 2A), whereas the ATP content fell from 3.8 ± 0.2 to 2.6 ± 0.1 μmol/g of cells within the same period (Figure 2B). In the presence of 1 mM fructose, no significant change in $[Mg^{2+}]_i$ or ATP was observed within 5 min of incubation (Figure 3). Incubation with 2–10 mM fructose increased $[Mg^{2+}]_i$ (Figure 3A), with a concomitant decrease in ATP content (Figure 3B), the maximal effect being reached with 3–10 mM fructose.

The time course of the effect of fructose on $[Mg^{2+}]_i$ was also studied within single hepatocytes by using dual excitation wavelengths. The ratio, R, of fluorescence at wavelengths 335 and 370 nm is independent of changes in the dye content of the cells [24]. Perifusing the cells with 10 mM fructose increased R by 16 % within 3 min (results not shown). This corresponds to a doubling of $[Mg^{2+}]_i$, thus confirming the results obtained with a single excitation wavelength. The dose response of $[Mg^{2+}]_i$ to fructose observed within single cells with dual excitation wavelengths (results not shown) was the same as in Figure 3(A).

$[Ca^{2+}]_i$ measurement

One difficulty of measuring $[Mg^{2+}]_i$ with mag-fura 2 is that the affinity of the probe for Ca^{2+} ($K_D = 53$ μM [2]) is much greater than for Mg^{2+} ($K_D = 1.5$ mM [2]). Taking into account the resting values for $[Ca^{2+}]_i$ in hepatocytes (approx. 0.2 μM [32–34]), increases in $[Ca^{2+}]_i$ might interfere with $[Mg^{2+}]_i$ measurements

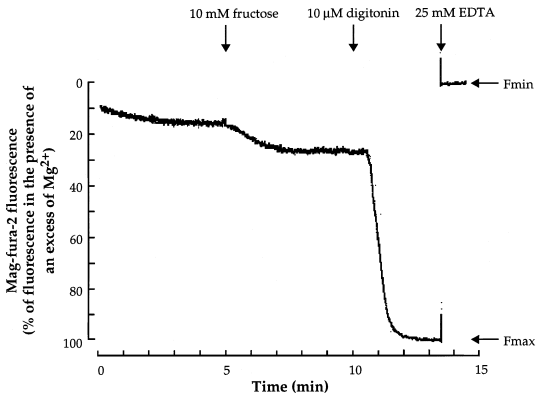

Figure 1 Time course of the effect of fructose on the fluorescence of mag-fura 2-loaded hepatocytes

Mag-fura 2-loaded hepatocytes were incubated in the presence of 10 mM glucose and, at the time indicated by the arrow, with 10 mM fructose. $[Mg^{2+}]_i$ was measured with a single excitation wavelength. The result of a representative experiment is shown. F_{max}, fluorescence in the presence of an excess of Mg^{2+} ions (100 %), was measured after the addition of digitonin; F_{min}, fluorescence in the absence of Mg^{2+} ions (0 %), was measured after the addition of EDTA.

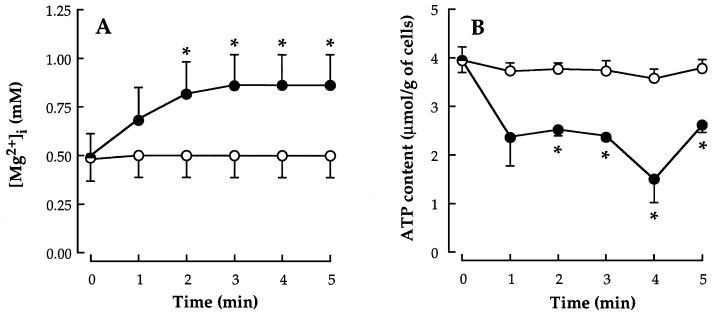

Figure 2 Time course of the effect of fructose on $[Mg^{2+}]_i$ (A) and ATP content (B)

In (**A**) the same protocol was used as that described in the legend to Figure 1. $[Mg^{2+}]_i$ was measured with a single excitation wavelength at the indicated times. In (**B**) hepatocytes were incubated for 15 min in the presence of 10 mM glucose. They were further incubated for the indicated times with 0.9% NaCl (control) or 10 mM fructose. The results are means ± S.E.M. for three different cellular preparations. *Significantly different ($P < 0.05$) from the respective control value. Symbols: ○, control; ●, 10 mM fructose.

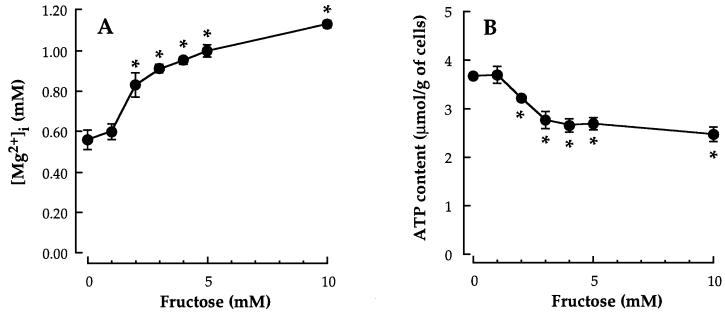

Figure 3 Dose-dependent effect of fructose on $[Mg^{2+}]_i$ (A) and ATP content (B)

In (**A**) the same protocol was used as that described in the legend to Figure 1 (5 min incubation with the indicated concentrations of fructose). $[Mg^{2+}]_i$ was measured with a single excitation wavelength. In (**B**) hepatocytes were incubated for 15 min in the presence of 10 mM glucose. They were incubated for a further 5 min with the indicated concentrations of fructose. The results are means ± S.E.M. for three different cellular preparations. *Significantly different ($P < 0.05$) from the respective control value. Symbols: ○, control; ●, 10 mM fructose.

[35]. Mag-fura 2 was indeed used to monitor Ca^{2+} oscillations in subcellular compartments of hepatocytes [36] or gastric epithelial cells [37]. We therefore studied whether fructose could modify hepatic $[Ca^{2+}]_i$ and whether an increase in $[Ca^{2+}]_i$ induced by vasopressin [32,33] interfered with $[Mg^{2+}]_i$ measurement. $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ were measured in fura 2- and mag-fura 2-loaded hepatocytes respectively (results not shown). Fructose (10 mM) did not significantly modify the fura 2 signal and hence did not alter $[Ca^{2+}]_i$. In contrast, $[Ca^{2+}]_i$ transients were observed with 50 nM vasopressin (from 0.20 ± 0.01 μM to 0.45 ± 0.05 μM, $n = 3$), as expected [32,33], and returned to resting values after 2 min. When added to mag-fura 2-loaded hepatocytes, vasopressin elicited a small transient decrease in fluorescence (results not shown). This would correspond to a 25% increase in $[Mg^{2+}]_i$ and could result either from influx of Mg^{2+} ions [7] or from the increase in $[Ca^{2+}]_i$ [32,33]. Fructose (10 mM) added before or after vasopressin nearly doubled $[Mg^{2+}]_i$ within 3 min (from 0.46 ± 0.07 mM to 0.80 ± 0.10 mM, $n = 7$).

Mg^{2+} ion efflux induced by fructose

Remarkably, the fructose-induced increase in $[Mg^{2+}]_i$ was much less than expected from the decrease in ATP content after 5 min of incubation. $[Mg^{2+}]_i$ increased only from 0.50 ± 0.11 to 0.86 ± 0.16 mM (i.e. from 0.25 to 0.43 μmol/g of cells, assuming that 1 g of cells contains approx. 0.5 ml of intracellular water [38]), whereas the ATP content decreased from 3.8 ± 0.2 to 2.6 ± 0.1 μmol/g of cells, thus releasing 1.2 ± 0.1 μmol of Mg^{2+} ions/g of cells. Mg^{2+} ions released from $MgATP^{2-}$ that do not appear as free Mg^{2+} ions could therefore be complexed to ligands other than ATP or be released into the extracellular medium. If all the Mg^{2+} ions released from $MgATP^{2-}$ (1.2 μmol/g of cells) were released into the extracellular medium, we should measure an increase in Mg^{2+} ions of 60 nmol/ml of incubation medium, i.e. 60 μM (for the cell concentration of 50 mg wet weight/ml used in this study). Because this amount was small compared with the Mg^{2+} ion concentration (1.2 mM) in the incubation buffer, cells were incubated in a Mg^{2+}-free buffer to measure Mg^{2+} efflux. We checked that the fructose-induced increase in $[Mg^{2+}]_i$ was similar in the absence or presence of extracellular Mg^{2+} ions (0.37 ± 0.05 mM in Mg^{2+}-supplemented buffer compared with 0.39 ± 0.03 mM in Mg^{2+}-free buffer; $n = 3$). The loss of Mg^{2+} ions induced by 10 mM fructose and measured in Mg^{2+}-free buffer was 0.35 ± 0.03 μmol/g of cells after 5 min of incubation. Similar results were obtained at 30 min (results not shown). This loss of Mg^{2+} ions represents approx. 30% of the loss expected (1.2 μmol/g of cells) if all the Mg^{2+} ions released from $MgATP^{2-}$ had been released into the extracellular medium. Therefore the major part of these Mg^{2+} ions must be sequestered inside the cells.

To challenge the buffer capacity of the cells towards Mg^{2+} ions, we inhibited the fructose-induced efflux of Mg^{2+} ions. Na^+ was replaced in the medium by choline to block Na^+/Mg^{2+} exchange [4], resulting in the complete inhibition of this efflux

Table 1 Effect of fructose on ATP content, $[Mg^{2+}]_i$ and extracellular Mg^{2+} ion concentration in hepatocytes incubated in Na^+ buffer or choline buffer

Hepatocytes were incubated, as described in the Experimental section, in Na^+ buffer or choline buffer. Fructose (10 mM) or 0.9% NaCl (control) was then added for 5 min. The difference in ATP content ($n = 3$), $[Mg^{2+}]_i$ ($n = 5$) and Mg^{2+} efflux ($n = 3$) between the control and the fructose condition is shown. Mg^{2+} ion efflux was measured in Mg^{2+}-free buffer. The results are means ± S.E.M. for the indicated number (n) of cellular preparations. *Significantly different ($P < 0.05$) from the value in Na^+ buffer. Abbreviation: n.d., not done.

Conditions	Decrease in ATP content (μmol/g of cells)	Increase in $[Mg^{2+}]_i$ (μmol/g of cells)	Increase in extracellular $[Mg^{2+}]$ (μmol/g of cells)
Na^+ buffer	1.2 ± 0.1	0.19 ± 0.02	0.35 ± 0.03
Choline buffer	n.d.	0.25 ± 0.02*	0.01 ± 0.01*

(Table 1). The increase in $[Mg^{2+}]_i$ induced by 10 mM fructose under this condition was slightly larger than that observed in cells incubated in Na^+ buffer (0.39 ± 0.03 mM in Na^+ buffer compared with 0.50 ± 0.03 mM in choline buffer; $n = 5$, $P < 0.05$).

Effects of fructose on the enzymes of glycogen metabolism

The time course of the activation of glycogen synthase and glycogen phosphorylase by 2 mM fructose was first studied. The activity of glycogen synthase increased progressively and reached a maximum at 20–30 min, whereas glycogen phosphorylase was transiently activated with a maximum reached within 5 min. The effects of 1–10 mM fructose on the activities of glycogen synthase and glycogen phosphorylase were therefore studied at 5 and 30 min of incubation (Figure 4). At 5 min of incubation no change in the activity of glycogen synthase was detected (Figure 4A), whereas glycogen phosphorylase was activated, the maximal effect being obtained with 3–10 mM fructose (Figure 4B). After a 30 min incubation, 1–5 mM fructose activated glycogen synthase, whereas no change was detected with 10 mM fructose (Figure 4A). At that time, only 3–10 mM fructose was activating glycogen phosphorylase (Figure 4B), but the extent of activation was smaller than at 5 min (Figure 4B). This difference in sensitivity towards fructose for glycogen synthase and glycogen phosphorylase activation was reflected in glycogen synthesis, which was stimulated by 1–3 mM fructose over a 30 min period, whereas no detectable effect was observed with concentrations higher than 5 mM (Figure 4C).

DISCUSSION

The first aim of this work was to study whether a fructose load could increase $[Mg^{2+}]_i$ as a consequence of ATP depletion. Incubation of hepatocytes with 3–10 mM fructose decreased ATP content by 30% (Figures 2B and 3B), nearly doubled $[Mg^{2+}]_i$ (Figures 2A and 3A) but did not change $[Ca^{2+}]_i$. Remarkably, the increase in $[Mg^{2+}]_i$ was less than expected from the decrease in ATP. An increase in $[Mg^{2+}]_i$, which was also smaller than expected from the decrease in ATP, had already been observed in opossum kidney cells subjected to anoxia [16]. The authors concluded that most of the Mg^{2+} ions released from $MgATP^{2-}$ were sequestered by ligands other than ATP or by intracellular stores. We studied whether a portion of these Mg^{2+} ions could also be released into the external medium, as suggested in [39]. From our measurements of $[Mg^{2+}]_i$ and of the Mg^{2+} ion loss from hepatocytes incubated in the presence of fructose, a balance sheet for the Mg^{2+} ions released from $MgATP^{2-}$ was calculated as follows (all values are in μmol/g of cells): (1) total quantity of Mg^{2+} ions expected to be released from $MgATP^{2-}$, 1.2; (2) amount lost in the extracellular medium, 0.35; (3) amount of Mg^{2+} released from $MgATP^{2-}$ and remaining in the cells, 0.85 [difference between (1) and (2)]; (4) actual increase in $[Mg^{2+}]_i$, 0.19; (5) amount of Mg^{2+} released from $MgATP^{2-}$ and buffered, 0.66 [difference between (3) and (4)]. Therefore 78% of the Mg^{2+} ions released from $MgATP^{2-}$ and remaining within the cells (0.66/0.85) were buffered. We challenged this buffer capacity by blocking the efflux of Mg^{2+} ions from fructose-treated hepatocytes, thus imposing on the cells an extra Mg^{2+} ion load of 0.35 μmol/g of cells. This resulted in an increase of $[Mg^{2+}]_i$ of 0.06 μmol/g of cells (see choline buffer compared with Na^+ buffer, in Table 1). Thus 0.29 μmol of Mg^{2+} ions (0.35 − 0.06), i.e. 83% of the extra Mg^{2+} ion load (0.29/0.35), have been buffered per g of cells. Although approx. 80% of the Mg^{2+} ions liberated from $MgATP^{2}$ are buffered, as much as 20% can appear as free Mg^{2+} ions and could act as a messenger by activating enzymes having a K_m for Mg^{2+} in the millimolar range.

The second aim of our study was to determine whether the increase in $[Mg^{2+}]_i$ induced by a fructose load could participate in the activation of glycogen synthase. In liver or muscle extracts, glycogen synthase phosphatase activity is stimulated (10–40%)

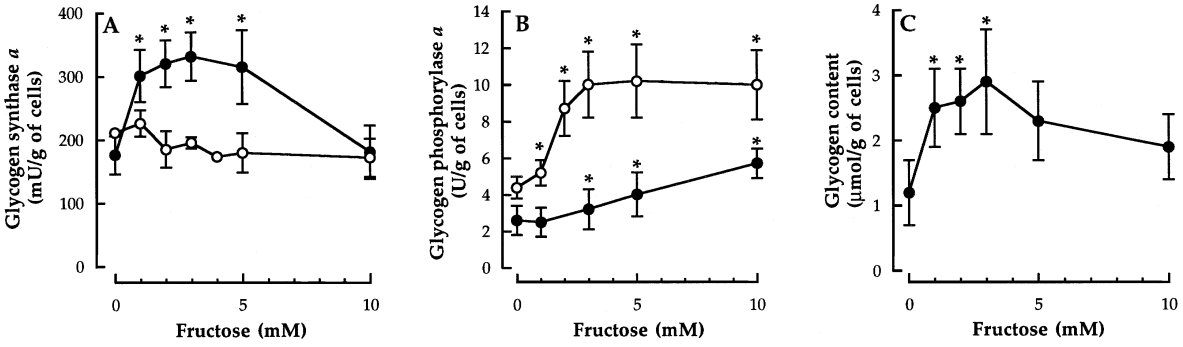

Figure 4 Dose-dependent effect of fructose on the activity of glycogen synthase (A) and glycogen phosphorylase (B), and on glycogen content (C)

Hepatocytes were incubated for 15 min in the presence of 20 mM glucose. They were incubated for a further 5 min (○) or 30 min (●) with the indicated concentrations of fructose. The results are means ± S.E.M. for at least three experiments. *Significantly different ($P < 0.05$) from the value with no addition.

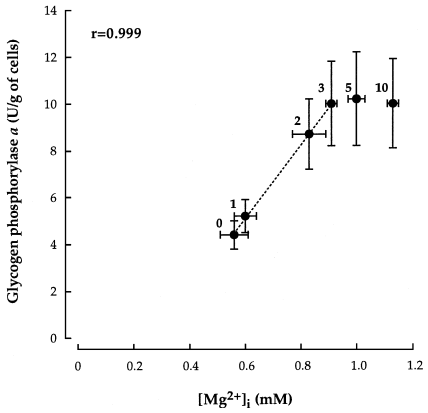

Figure 5 Correlation between glycogen phosphorylase activity and $[Mg^{2+}]_i$

Glycogen phosphorylase activity at 5 min of incubation in the presence of increasing concentrations of fructose (Figure 4B) was correlated with the increase in $[Mg^{2+}]_i$ observed under the same conditions (Figure 3A). The linear regression was calculated for fructose concentrations ranging from 0 to 3 mM. The concentrations of fructose (mM) are indicated above each symbol.

by 1 mM $MgCl_2$, depending on the type of phosphatase involved and the site(s) of glycogen synthase dephosphorylated [40]. In hepatocytes incubated with 3 mM fructose, $[Mg^{2+}]_i$ reached nearly 1 mM (Figure 3A), suggesting that Mg^{2+} might be involved in the activation of glycogen synthase observed in this condition (Figure 4A). Our data do not support this hypothesis. At 1 mM fructose, glycogen synthase was activated, whereas no change in $[Mg^{2+}]_i$ was detectable. Conversely, at 10 mM fructose, $[Mg^{2+}]_i$ was increased but no activation of glycogen synthase was detected. Clearly the fructose-induced activation of glycogen synthase cannot be related to an increase in $[Mg^{2+}]_i$. As to the mechanism involved in glycogen synthase activation by fructose, we can only speculate. A glucose-6-phosphate-mediated mechanism deserves consideration. Indeed, it has already been proposed for the fructose-induced activation of glycogen synthase [41] and is probably involved in the activation of the enzyme by glucose [42].

If we can exclude the mediation of $[Mg^{2+}]_i$ in glycogen synthase activation, we cannot do so for glycogen phosphorylase activation. Figure 5 shows a correlation ($r = 0.999$) between the increase in $[Mg^{2+}]_i$ and the activation of glycogen phosphorylase when hepatocytes were incubated with 0–3 mM fructose for 5 min. This is in agreement with a previous suggestion that an increase in $[Mg^{2+}]_i$ might mediate the activation of glycogen phosphorylase through a stimulation of phosphorylase kinase [43].

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