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Culture duration and conditions affect the oscillations of cytoplasmic calcium concentration induced by glucose in mouse pancreatic islets

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Summary The pattern of the increase in cytoplasmic Ca_i^{2+} that glucose produces in beta cells has been reported to be highly variable. Here, we evaluated the influence of the culture duration (1–4 days) and conditions (5–10 mmol/l glucose) on Ca_i^{2+} in normal mouse islets stimulated by glucose. After 1 day of culture in 10 mmol/l glucose, a rise of the glucose concentration from 3 to 15 mmol/l induced a triphasic change of Ca_i^{2+} in the islets. A small initial decrease was followed by a large peak increase and then by regular fast oscillations (~2.5/min). When the culture was prolonged to 2, 3 and 4 days, the initial decrease became inconsistent and the peak occurred earlier, whereas the oscillations decreased in frequency, increased in duration and eventually disappeared; on day 4 the Ca_i^{2+} rise was sustained. After culture in 5 mmol/l glucose, the pattern of Ca_i^{2+} changes induced by 15 mmol/l glucose was different. The initial decrease was very pronounced, the first peak was delayed and clearly separated from the subsequent oscillations. These were of a mixed type (fast Ca^{2+} transients on top of slow ones) after 1 day, and of a slow type only after 4 days. These alterations in the Ca_i^{2+} oscillations triggered by glucose could not be ascribed to desynchronization of the signal between different regions of the islets. In conclusion, culturing normal mouse islets in 5 or 10 mmol/l glucose for 1–4 days, markedly alters the characteristics of the changes in Ca_i^{2+} produced by glucose. This pitfall must be borne in mind when studying stimulus-secretion coupling in beta cells from normal or diabetic animals, or from human islets. [Diabetologia (1994) 37: 1007–1014]

Key words Islets, culture, calcium, glucose, stimulus-secretion coupling, beta cells.

The essential role of Ca^{2+} in the triggering of insulin secretion has been established beyond doubt [1–5]. Glucose and most other nutrient secretagogues promote Ca^{2+} influx in pancreatic beta cells. This influx occurs rhythmically during phases of electrical activity corresponding to slow waves of membrane depolarization and opening of voltage-dependent Ca^{2+} channels [4, 6, 7].

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Abbreviation: Ca_i^{2+}, concentration of cytoplasmic calcium.

Since the development of fluorimetric methods to measure the concentration of free ions in intact cells, a number of studies have investigated the effects of various secretagogues on cytoplasmic Ca_i^{2+} in insulin-secreting cells. Suspensions of normal mouse [8] or rat [9] islet cells, of RINm5F cells [10] or of rat islets [11] were first used. Relatively straightforward conclusions could be drawn from the average changes that were recorded, in particular after the technique was improved by the use of dual-wavelength fluorometry [12, 13]. With the further development of microtechniques and their application to the measurements of Ca_i^{2+} in single islets or single beta cells, new questions have been raised which currently limit the significance of the observations. From studies using isolated rat or mouse beta cells cultured for 1 to 5 days [14–20], two conclusions have emerged. First,

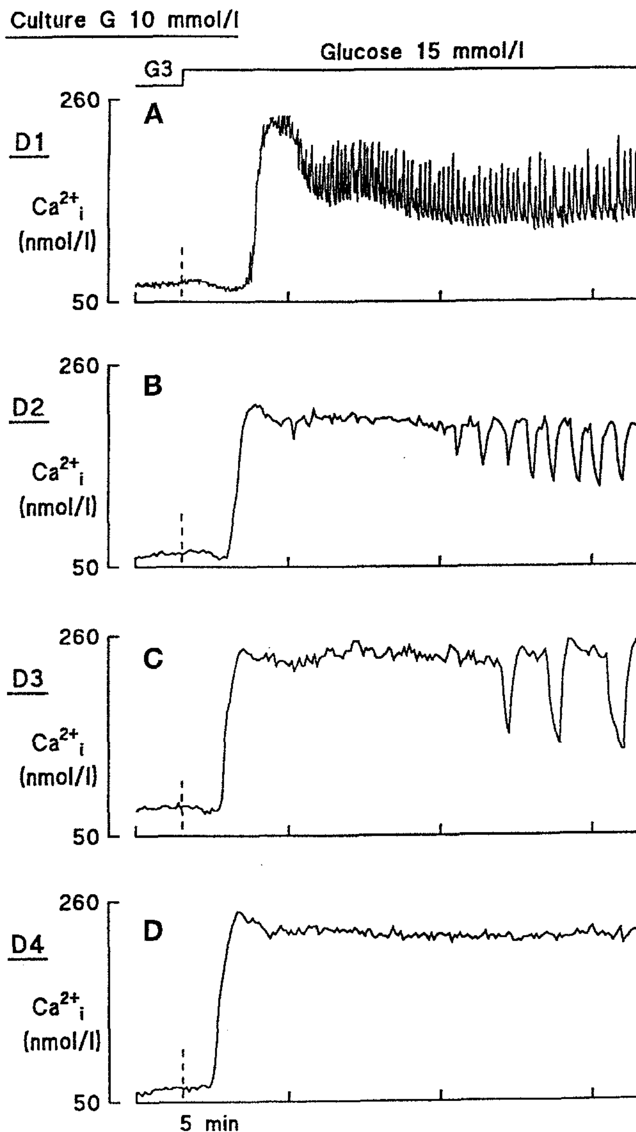

Fig. 1 A–D. Influence of culture duration on the changes in Ca_i^{2+} occurring in mouse islets stimulated by an increase in glucose (G) concentration from 3 to 15 mmol/l. The islets were cultured in RPMI 1640 medium containing 10 mmol/l glucose for 1–4 days (D1–D4). They were then loaded with fura-2, transferred to the recording chamber, and perifused with a medium containing 3 mmol/l glucose for about 10 min before Ca_i^{2+} measurements started. These recordings are representative of results obtained in 18 (D1) or 12 (D2–D4) islets

there exists a marked variability of the individual Ca_i^{2+} responses to glucose. Second, Ca_i^{2+} oscillations are present in single beta cells stimulated by a constant concentration of glucose, but their frequency is highly variable and much lower (0.05–0.5/min) than that of the slow waves of membrane potential. These findings are in contrast with those obtained with mouse islets a few hours after isolation [21, 22] or after overnight culture [23, 24]. In this model, the Ca_i^{2+} changes in response to glucose were found to be more homogeneous and to display oscillations with a similar frequency to those of the oscillations of membrane potential recorded in beta cells [22, 24]. However, in one study with rat islets cultured for 2–4 days, only slow oscillations of Ca_i^{2+} were detected [25].

As a first step to understanding these discrepancies, we evaluated the influence of the culture duration (1–4 days) and of the glucose concentration during culture (5 or 10 mmol/l) on Ca_i^{2+} changes in normal mouse islets.

Materials and methods

Preparation and solutions. Islets were isolated aseptically after collagenase digestion of the pancreas of fed female NMRI mice (25–30 g). They were then cultured for 1–4 days in RPMI 1640 medium (Flow Laboratories, ICN Biomedicals Ltd, Irvine, UK, or Sera Lab Ltd, Crawley Down, Sussex, UK) containing 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. The concentration of glucose was 5 or 10 mmol/l. The culture medium was changed after 2 days.

The medium used for the isolation of islets and for all experiments was a bicarbonate-buffered solution containing: (in mmol/l) 120 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$ and 24 $NaHCO_3$. It was gassed with O_2/CO_2 (94/6) to maintain pH 7.4, and was supplemented with 1 mg/ml bovine serum albumin (fraction V, Boehringer Mannheim, Mannheim, Germany).

Measurements of cytoplasmic Ca_i^{2+}. Cultured islets were loaded with fura-2 during 40 min of incubation at 37 °C in a bicarbonate-buffered solution containing 10 mmol/l glucose and 1 μmol/l fura-2-acetoxymethylester (Molecular Probes, Eugene, Or., USA; added from a 1 mmol/l stock solution in Me_2SO). In one series of control experiments, islets cultured in the presence of 5 mmol/l glucose were loaded with fura-2 in a medium also containing 5 mmol/l glucose. These islets were not included in the quantification of Ca_i^{2+} changes. Islets of a similar, medium, size were used for all experiments. Large islets were not used because they survive less well in culture and inhomogeneously take up fura-2. Loaded islets were then transferred to a temperature-controlled perifusion chamber (Applied Imaging, Sunderland, UK) with a volume of approximately 1 ml and a bottom made of a coverslip. The chamber was mounted on the stage of an inverted microscope. The islets were held in place by gentle suction with a glass micropipette. The chamber was perfused at a flow rate of 1.3 ml/min. The dead space of the system (2 min) has been corrected for in figures and calculations. Perifusion solutions were kept at 38 °C in a water bath and the temperature controller ensured a temperature of 37 °C (± 0.3 °C) in the chamber as monitored by a thermistor placed near the tissue.

The measurements of Ca_i^{2+} were performed with the system MagiCal (Applied Imaging) as recently described in detail [24]. The tissue was excited at 340 and 380 nm. The fluorescence emitted at 510 nm was captured by a CCD video camera (Photonic Science Ltd, Tunbridge Wells, UK). The time interval between successive series of 340–380 images was usually 3.3 s, which limited the duration of the experiments to 14 min. For longer experiments (up to 30 min), the fluorescence was recorded on video tape (Sony VO-9600P). From the ratio of the fluorescence at 340 and 380 nm, the concentration of Ca_i^{2+} was calculated by comparison with a calibration curve [24].

Presentation of results. All measurements of Ca_i^{2+} (calculated concentrations) are illustrated by recordings which are representative of results obtained with the indicated number of is-

Table 1. Influence of culture conditions on Ca_i^{2+} changes occurring in mouse islets stimulated by an acute rise in glucose concentration from 3 to 15 mmol/l

Line	Measured parameter	Culture in 10 mmol/l glucose				Culture in 5 mmol/l glucose	
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 4
(1)	Ca_i^{2+} in G3 (nmol/l)	83 ± 4	70 ± 5	76 ± 5	74 ± 3	92 ± 5	66 ± 3[b]
(2)	Islets in which G15 induced an initial decrease in Ca_i^{2+}	37/38	7/12	8/12	9/25	14/14	12/12
(3)	Initial decrease in Ca_i^{2+} induced by G15 (nmol/l)	6.3 ± 0.4	6.9 ± 1.2	5.1 ± 0.2	4.2 ± 0.4	11.0 ± 0.8[d]	13.0 ± 0.8[d]
(4)	Delay of the increase in Ca_i^{2+} induced by G15 (s)	92 ± 3	87 ± 6	68 ± 4[b]	55 ± 4[b]	157 ± 4[d]	131 ± 7[b,d]
(5)	Ca_i^{2+} at the first peak in G15	215 ± 7	217 ± 11	259 ± 9[a]	248 ± 9[a]	186 ± 6[c]	198 ± 7[d]
(6)	Delay to first peak of Ca_i^{2+} (s)	144 ± 2	142 ± 6	118 ± 5[b]	106 ± 4[b]	193 ± 3[d]	169 ± 8[b,d]
(7)	Rate of Ca_i^{2+} rise (nmol/s)	2.6 ± 0.1	2.8 ± 0.3	3.7 ± 0.3[b]	3.4 ± 0.2[b]	2.5 ± 0.2	3.6 ± 0.4[b]
(8)	Islets showing Ca_i^{2+} oscillations during stimulation by G15	38/38	12/12	9/12	8/25	14/14	12/12
(9)	Average Ca_i^{2+} (nmol/l) between 9–12 min in G15	156 ± 4	167 ± 8	203 ± 5[b]	211 ± 7[b]	125 ± 5[d]	115 ± 4[d]

Values are means ± SEM for the indicated number of islets. The islets were cultured for 1–4 days in RPMI medium containing either 10 or 5 mmol/l glucose before Ca_i^{2+} was measured as follows. After loading for 40 min in a bicarbonate-buffered solution containing 10 mmol/l glucose and 1 μmol/l Fura-2-AM, the islets were perifused for ~ 10 min with a medium containing 3 mmol/l glucose (G3). Thereafter, the concentration of glucose was raised to 15 mmol/l (G15). An initial decrease in Ca_i^{2+} was considered to be present if larger than 2 nmol/l; its mean size was calculated only for those islets in which it was present. The delay of the increase in Ca_i^{2+} was calculated at the time when Ca_i^{2+} was 5 nmol/l higher than in 3 mmol/l glucose. The average Ca_i^{2+} concentration was calculated by integrating all Ca_i^{2+} values between 9 and 12 min of stimulation with 15 mmol/l glucose. [a] $p < 0.05$, [b] $p < 0.01$ or less vs day 1 for islets cultured at the same glucose concentration; [c] $p < 0.05$, [d] $p < 0.01$ or less vs islets cultured in 10 mmol/l glucose for the same period.

lets. For each culture, usually three islets were tested with the same protocol each day. A given protocol was tested in islets from at least four different cultures. The statistical significance between means was assessed either by Student's *t*-test for paired or unpaired values when only two groups were compared. For more than two groups, analysis of variance followed by Scheffé's test was used. Differences were considered statistically significant at *p* less than 0.05.

Results

Islets cultured in 10 mmol/l glucose. After their transfer into the chamber, the islets loaded with fura-2 were perifused for about 10 min with a medium containing 3 mmol/l glucose. Under these conditions, Ca_i^{2+} was low and stable (Fig. 1), and not significantly affected by the duration of culture (Table 1, line 1). When the concentration of glucose was raised from 3 to 15 mmol/l, a concentration that causes half-maximal stimulation of mouse beta cells [23], Ca_i^{2+} in islet cells changed in three phases: an initial small decrease, followed by a marked increase and eventually a more or less sustained elevation.

The initial decrease was almost always observed on day 1 of culture, but its occurrence became less frequent as the culture was prolonged. When it was present, its amplitude tended to decrease after day 2, but this trend was not statistically significant (Table 1, lines 2 and 3).

The initial increase in Ca_i^{2+} was similar in onset and amplitude after 1 and 2 days of culture. After 3 and 4 days, however, the increase occurred earlier and was larger than after 1 day; the rate of Ca_i^{2+} rise was also higher (Table 1, lines 4–7).

After the first rise, Ca_i^{2+} remained elevated, but the pattern was strikingly different after 1 to 4 days of culture (Fig. 1). After 1 day, rapid and fairly regular oscillations of Ca_i^{2+} were recorded in all islets. Their frequency was typically higher during the first minutes than under steady-state stimulation (Fig. 1 A). On days 2 and 3, much slower oscillations occurred relatively late during the stimulation. They were seen in all islets on day 2, and in 9 of 12 islets on day 3. On day 4, most islets showed a sustained rise of Ca_i^{2+} (Fig. 1 D); oscillations were present in only 8 of 25 islets, and their presence was often transient. The average Ca_i^{2+} level (integrated between 9 and 12 min of stimulation with 15 mmol/l glucose) clearly increased with the duration of culture because of the disappearance of the oscillations (Table 1, line 9).

The characteristics of Ca_i^{2+} oscillations occurring during steady-state stimulation were studied in islets perifused with 15 mmol/l glucose throughout the experiment. These oscillations are illustrated in Figure 2 and quantified in Table 2. On day 1, all islets displayed rapid and fairly regular Ca_i^{2+} oscillations (Fig. 2 A). In some islets, however, a slower periodicity marked by deeper and longer decreases in Ca_i^{2+} was

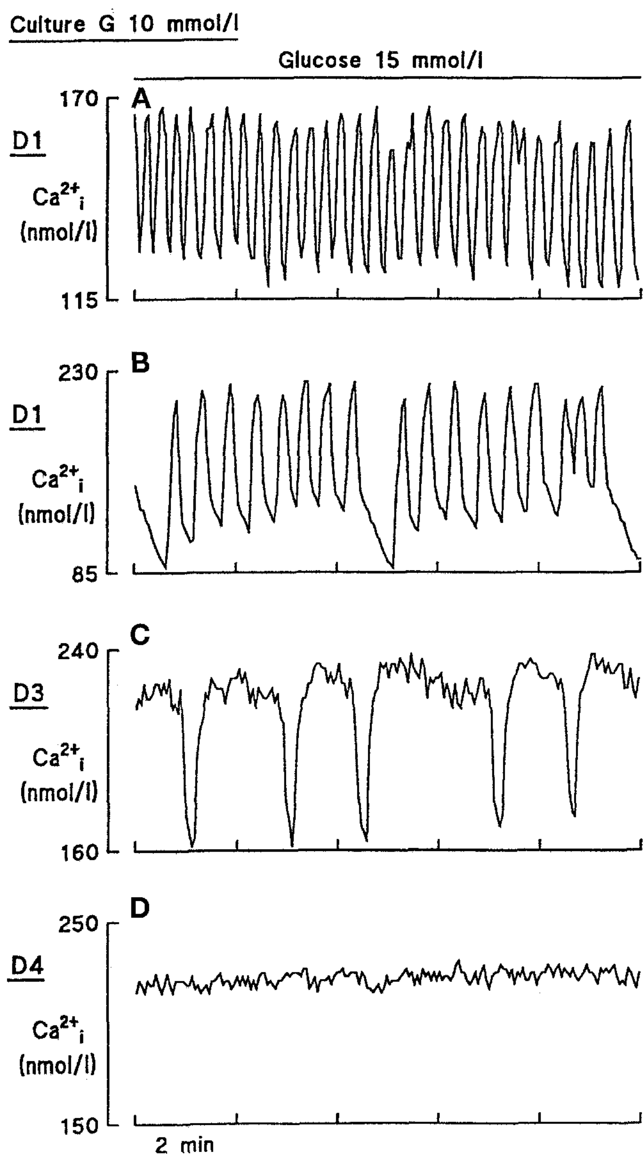

Fig. 2 A–D. Influence of culture duration on Ca_i^{2+} oscillations recorded in mouse islets during steady-state stimulation with 15 mmol/l glucose. The islets were cultured in RPMI 1640 medium containing 10 mmol/l glucose for 1, 3 or 4 days (D1, D3 and D4). They were then loaded with fura-2, transferred to the recording chamber, and perifused with a medium containing 15 mmol/l glucose for about 12 min before Ca_i^{2+} measurements started. Note that the scale for Ca_i^{2+} is not the same in the different panels. These recordings are representative of results obtained in 12 islets (D1 and D3) or 15 islets (D4)

also observed (Fig. 2 B). On day 2, Ca_i^{2+} oscillations were consistently present, but their frequency was much lower than on day 1 (Table 2). On day 3, the proportion of islets with Ca_i^{2+} oscillations decreased, but the frequency of these oscillations was similar to that of islets on day 2. There were no Ca_i^{2+} oscillations on day 4. Table 2 (line 3) also shows that the average Ca_i^{2+} level (integrated over the 14 min of recording regardless of the presence of oscillations) increased with the duration of culture.

We have previously reported that Ca_i^{2+} oscillations are synchronous in all subregions of islets stimulated by 15 mmol/l glucose after 1 day of culture in 10 mmol/l glucose [24]. In the 7 of 12 islets which displayed oscillations after 3 days of culture (Table 2, line 1), the oscillations were also found to be synchronous in all subregions. In the other five islets no oscillations were detected in any subregion. Similarly, in six islets which did not show Ca_i^{2+} oscillations after 4 days of culture, the Ca_i^{2+} signal was also found to be stable when analysed in subregions.

Experiments were also performed with a Ca^{2+}-free medium. In the presence of 3 mmol/l glucose, the average Ca_i^{2+} concentration was 38 ± 3 nmol/l and 43 ± 2 nmol/l after 1 and 4 days of culture, respectively. Stimulation by 15 mmol/l glucose similarly and significantly ($p < 0.001$ by paired t-test; $n = 8$) lowered Ca_i^{2+} in both series of islets, by 4.2 ± 0.2 nmol/l and by 4.6 ± 0.6 nmol/l, respectively.

Islets cultured in 5 mmol/l glucose. In the presence of 3 mmol/l glucose, Ca_i^{2+} was lower in islets cultured for 4 days than in islets cultured for 1 day only, but there was no significant difference with islets cultured for the same period of time in a medium containing 10 mmol/l glucose (Table 1, line 1).

When the glucose concentration was raised from 3 to 15 mmol/l, Ca_i^{2+} again changed in three phases (Fig. 3). The initial fall was much larger than after culture in 10 mmol/l glucose, and was seen in all islets, even after 4 days of culture (Table 1, lines 2 and 3). The subsequent increase in Ca_i^{2+} occurred slightly sooner after 4 days than after 1 day of culture, but reached a similar peak value. Compared with islets cultured in 10 mmol/l glucose, the Ca_i^{2+} rise was delayed and its amplitude was smaller (Table 1, lines 4–7).

Subsequently, Ca_i^{2+} started to oscillate following different patterns (Fig. 3). On day 1, relatively rapid oscillations of small amplitude appeared for a few min before being replaced by much larger and slower oscillations. This pattern was observed in most of the islets, whether the loading with fura-2 was performed in the presence of 10 or 5 mmol/l glucose (compare Fig. 3 A and B). In a minority of islets Ca^{2+} remained elevated without oscillations between the initial large peak and the slow oscillations. After 4 days of culture, only slow Ca_i^{2+} oscillations were seen (Fig. 3 C).

The Ca_i^{2+} oscillations that occurred during steady-state stimulation with 15 mmol/l glucose are shown in greater detail in Figure 4. It is evident that they were markedly different after 1 and 4 days of culture. After 1 day, they were complex, often composed of slow, ample fluctuations of Ca_i^{2+} and of fast, smaller changes (Table 2). These faster oscillations were either present all the time or only on top of the slow oscillations (Fig. 4 A and B). On the other hand, the oscillations were always slow and simple after

Table 2. Influence of culture conditions on the characteristics of Ca_i^{2+} in mouse islets during continuous perifusion with a medium containing 15 mmol/l glucose

Line	Measured parameter	Culture in 10 mmol/l glucose				Culture in 5 mmol/l glucose	
		Day 1	Day 2	Day 3	Day 4	Day 1	Day 4
(1)	Proportion of islets with Ca_i^{2+} oscillations	12/12	12/12	7/12	0/15	14/14	18/18
(2)	Frequency of the Ca_i^{2+} oscillations (per min)						
	Fast	2.6 ± 0.4	1.0 ± 0.2[b]	0.9 ± 0.2[b]	0	2.5 ± 0.2 (11)	0
	Slow					0.21 ± 0.02 (12)	0.38 ± 0.05[b] (18)
(3)	Average Ca_i^{2+} (nmol/l)	145 ± 9	162 ± 6	192 ± 11[a]	217 ± 12[b]	125 ± 6[c]	116 ± 7[d]

Values are means ± SEM for the indicated number of islets. After culture and loading with fura-2, the islets were immediately and continuously perifused with a medium containing 15 mmol/l glucose. The measurements of Ca_i^{2+} started about 12 min after the start of this perifusion. On day 3 of the culture in 10 mmol/l glucose, the frequency of Ca_i^{2+} oscillations was calculated only for those 7 islets displaying oscillations. On day 1 of the culture in 5 mmol/l glucose, 9 islets showed mixed (fast and slow) oscillations, 2 islets only fast oscillations, and 3 islets only slow oscillations. [a] $p < 0.05$, [b] $p < 0.01$ or less vs day 1 for islets cultured at the same glucose concentration; [c] $p < 0.05$, [d] $p < 0.01$ or less vs islets cultured in 10 mmol/l glucose for the same period

4 days (Fig. 4 C and D). The average Ca_i^{2+} level during glucose stimulation was not different between days 1 and 4, but clearly lower than in islets cultured for the same time in 10 mmol/l glucose (Tables 1 and 2).

We also investigated whether the mixed oscillations of Ca_i^{2+} recorded after culture of the islets in 5 mmol/l glucose were synchronous in all subregions of the organ. After 1 day of culture, a good synchrony of both slow and fast Ca_i^{2+} oscillations was observed in 14 of 14 islets stimulated by 15 mmol/l glucose throughout (Fig. 5 A). On the other hand, *slight* desynchronization of Ca_i^{2+} oscillations was observed in 7 of 18 islets cultured for 4 days in 5 mmol/l glucose. In the islet shown in Figure 5 B, five oscillations occurred in region 5 and only four in quarters 2 and 3 during the period of observation. Although the desynchronization was limited in this example, it is the most striking one that we have observed.

Discussion

The present study shows that the characteristics of glucose-induced Ca_i^{2+} changes in normal mouse islets are markedly modified by the duration of the culture and by the concentration of glucose during the culture period. It has previously been established that, although islets from normal mice contain about 20 % of non-beta cells, the characteristics of the Ca_i^{2+} signals recorded from the microorgan essentially reflect events occurring in beta cells [21, 22, 24, 26].

Acute stimulation of the islets by a rise of the glucose concentration from 3 to 15 mmol/l produced an initial lowering of Ca_i^{2+} which has been observed in other studies [8, 13, 19, 24, 27]. This decrease is thought to reflect Ca^{2+} sequestration in cellular organelles, but its functional significance is unclear. It was most pronounced after culture in 5 mmol/l glucose, and became smaller and inconsistent when the culture in 10 mmol/l glucose was extended beyond 2 days. We attribute this apparent disappearance to a masking by the faster subsequent rise in Ca_i^{2+}. Thus, when experiments were performed in the absence of extracellular Ca^{2+}, glucose similarly lowered Ca_i^{2+} after 1 and 4 days of culture. These observations are in agreement with the report that the initial lowering of Ca_i^{2+} was more easily detected when dispersed islet cells were stimulated by glucose after preincubation in 3 rather than 20 mmol/l glucose [13].

The initial small decrease was followed by a large first phase increase of Ca_i^{2+}, which was little affected by culture conditions and duration. The major impact of culture was on Ca_i^{2+} oscillations occurring during steady-state stimulation with glucose. Depending on the conditions, these oscillations progressively disappeared or changed in shape and frequency. Elucidation of the causes of the complex changes in Ca_i^{2+} responses induced by culture is not easy and would first require identification of the regulatory mechanisms that control Ca_i^{2+} oscillations. However, some conclusions can already be drawn from our observations.

In both freshly isolated islets [22] and in islets cultured for only 1 day in 10 mmol/l glucose [24], fast Ca_i^{2+} oscillations are synchronous in all subregions of the islets and display the same characteristics as the oscillations of beta-cell membrane potential. Glucose-induced electrical activity is altered in single mouse beta cells [28, 29], which might have to be electrically coupled in order to display regular slow waves [30]. A regular pattern of fast Ca_i^{2+} oscillations has never been described in single mouse beta cells cultured for 1–2 days in RPMI medium with 11 mmol/l glucose and then stimulated by various concentrations of the sugar. They usually display Ca_i^{2+} oscillations with a slow frequency of 0.05–0.5/min [18], similar to the slow oscillations that we observe in islets cultured for 4 days in 5 mmol/l glucose. We therefore investigated whether the changes in the Ca_i^{2+} pat-

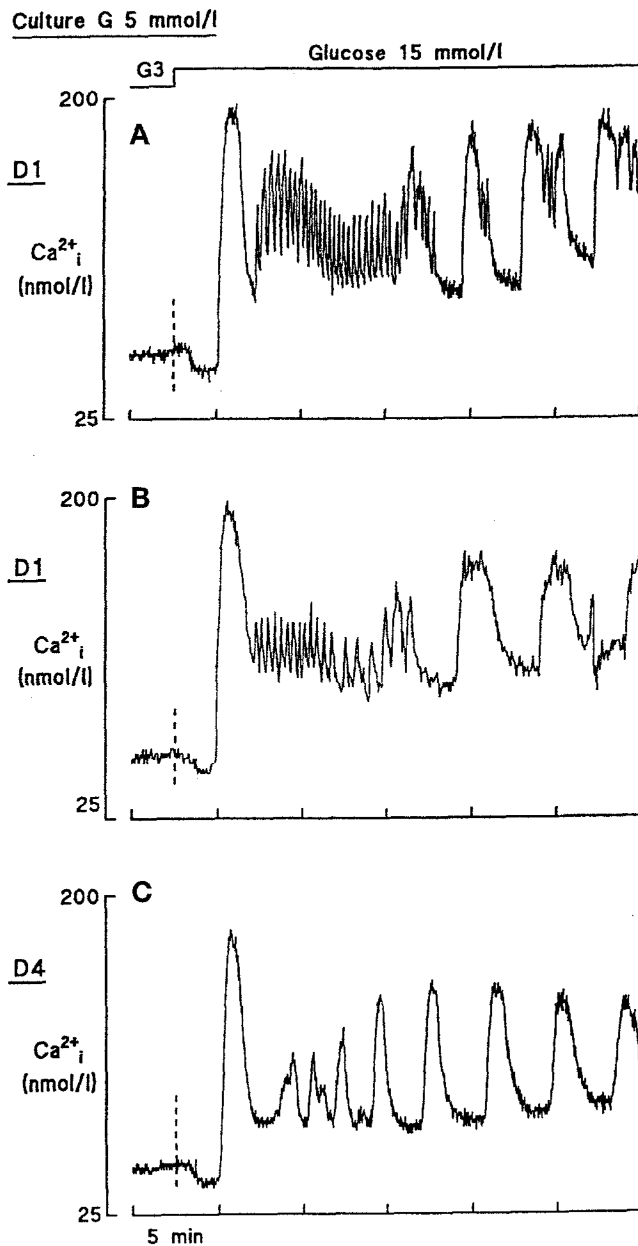

Fig. 3 A–C. Influence of culture duration on the changes in Ca_i^{2+} occurring in mouse islets stimulated by an increase in glucose (G) concentration from 3 to 15 mmol/l. The islets were cultured in RPMI 1640 medium containing 5 mmol/l glucose for 1 or 4 days (D1 and D4). They were then loaded with fura-2 in the presence of 10 mmol/l glucose (A and C) or 5 mmol/l glucose (B). After their transfer to the recording chamber, the islets were perifused with a medium containing 3 mmol/l glucose for about 10 min before Ca_i^{2+} measurements started. These recordings are representative of results obtained in 11, 6 and 9 islets, respectively

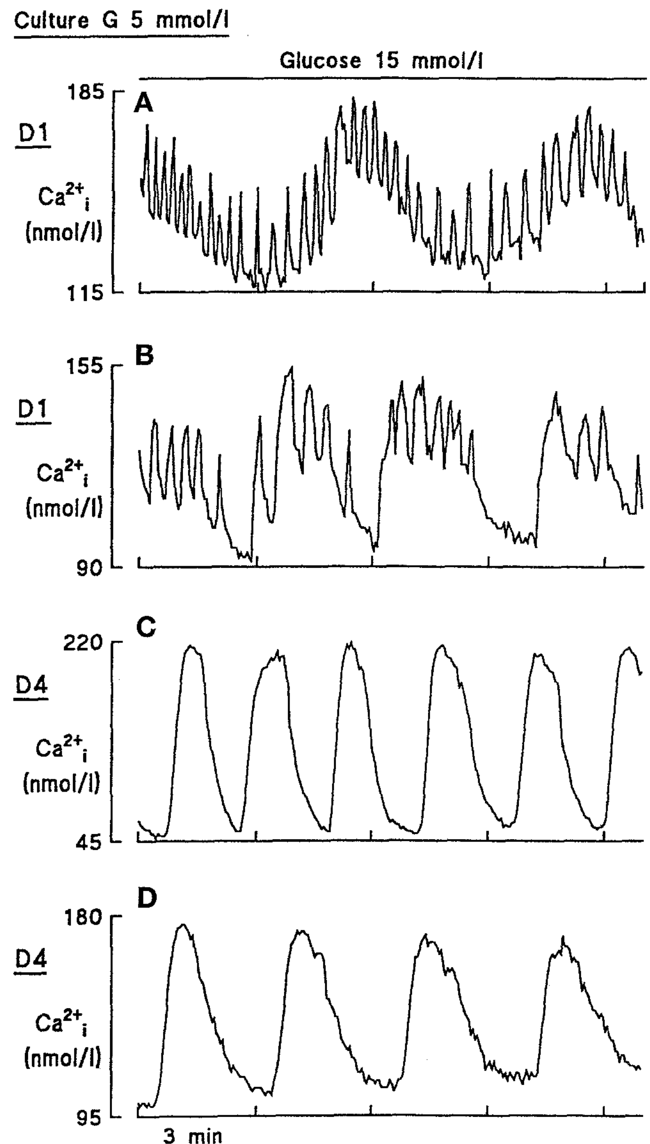

Fig. 4 A–D. Influence of culture duration on Ca_i^{2+} oscillations recorded in mouse islets during steady-state stimulation with 15 mmol/l glucose. The islets were cultured in RPMI 1640 medium containing 5 mmol/l glucose for 1 or 4 days (D1 and D4). They were then loaded with fura-2, transferred to the recording chamber, and perifused with a medium containing 15 mmol/l glucose for about 12 min before Ca_i^{2+} measurements started. Note that the scale for Ca_i^{2+} is not the same in the different panels. These recordings are representative of results obtained in 14 and 18 islets, respectively

tern observed during culture could be due to desynchronization of the oscillations occurring in different regions of the islets. This, however, could not explain the loss of Ca_i^{2+} oscillations occurring during culture in 10 mmol/l glucose or the appearance of mixed oscillations (fast Ca_i^{2+} transients on top of slower ones) after 1 day of culture in 5 mmol/l glucose. Even after 4 days of culture in 5 mmol/l glucose, most of the islets maintained good synchrony between all regions studied. Only in a few islets did we find partial desynchronization. We do not believe, therefore, that a loss of beta cell synchrony is the primary cause of the changes of Ca_i^{2+} pattern occurring under our culture conditions. That other factors are involved is indirectly supported by the observation that mixed oscillations occurred in a higher proportion of single beta cells when these were studied just after isolation than after 1–2 days of culture [18].

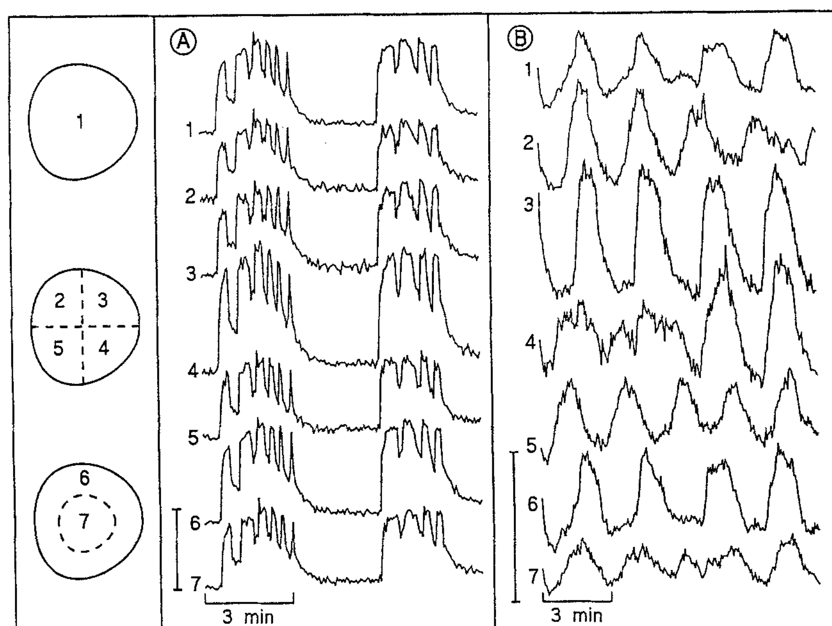

Fig. 5 A, B. Influence of culture duration on the synchrony of Ca_i^{2+} oscillations in various regions of islets stimulated by 15 mmol/l glucose. The mean Ca_i^{2+} was determined in the whole islet (1) or in the subregions (2–7). The islets were cultured in RPMI 1640 medium containing 5 mmol/l glucose for 1 day (**A**) or for 4 days (**B**). The vertical calibration bars correspond to 100 nmol/l Ca_i^{2+}

The changes in glucose metabolism that take place in islet cells during culture at various glucose concentrations [31, 32] are likely to play some role. The progressively more rapid and sustained elevation of Ca_i^{2+} after longer culture in 10 mmol/l glucose might be a consequence of the acceleration of glucose metabolism under these conditions. This interpretation is compatible with our unpublished observations that 1) slow Ca_i^{2+} oscillations reappeared in islets cultured for 4 days when the concentration of glucose was reduced from 15 to 10 mmol/l in the perifusion medium and 2) culture barely affected the Ca_i^{2+} changes induced by arginine, an amino acid which does not affect beta cell function through changes in metabolism [33]. The observations that the Ca_i^{2+} rises brought about by arginine or by glucose (during both first and second phase) were not attenuated by 4 days of culture also speak against the possibility that culture markedly affected voltage-dependent Ca^{2+} channels.

The present study raises the question of what is the normal pattern of glucose-induced Ca_i^{2+} changes in beta cells. For the mouse beta cell, the pattern that we observed after no more than 1 day of culture in 10 mmol/l glucose is similar to that observed by others in non-cultured islets [21, 22]. It also closely resembles the changes in beta cell membrane potential (more specifically of the Ca^{2+}-dependent electrical activity) recorded in islets still within the pancreas and studied rapidly after the death of the animal [4, 24]. In a minority of freshly isolated islets, 11 mmol/l glucose produced mixed Ca_i^{2+} oscillations or slow Ca_i^{2+} oscillations [21] similar to those observed here after 1 and 4 days of culture in 5 mmol/l glucose, respectively. We did not record the purely slow pattern in islets cultured for 1 day in 10 mmol/l glucose and subsequently challenged by 15 mmol/l glucose. However, a mixed pattern was occasionally observed, and may be more frequent during stimulation by 10 than 15 mmol/l [34 and our unpublished data]. It is possible that these mixed Ca_i^{2+} oscillations correspond to the periodic electrical activity that is sometimes recorded in fresh islets [35]. In intact rat islets cultured for 2–4 days in the presence of 10 mmol/l glucose, acute challenge with 10 mmol/l glucose induced slow Ca_i^{2+} oscillations with a frequency of about 0.25 per min [25]. No fast oscillations have been described in that study with rat islets, but they could not have been detected by sampling images every 20–30 s only [25].

The most important message of our work is that great caution should be exercised when drawing conclusions from Ca_i^{2+} measurements obtained in beta cells or in islets cultured for several days. In addition to the actual conditions of measurements both culture duration and conditions may affect the responses. This complicates the analysis of the abnormalities of stimulus-secretion coupling in beta cells from animal models of diabetes, and adds to the difficulties of studying human beta cells.

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