Synchronous glucose-dependent $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets of Langerhans recorded in vivo

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Abstract Using microfluorescence in combination with image-analysis techniques we monitored intracellular calcium ($[Ca^{2+}]_i$) dynamics in mouse islets of Langerhans loaded with fura-2 and recorded in vivo. $[Ca^{2+}]_i$ oscillates in the glycaemias range 5–10 mM, the duration of the oscillations being directly proportional to the blood glucose concentration. The analysis of different areas within the same islet shows that $[Ca^{2+}]_i$ oscillations are synchronous throughout the islet. These results show that in vivo, individual islets of Langerhans behave as a functional syncytium and suggest the existence of secretory pulses of insulin. **© 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Ca^{2+}; Pancreas; Oscillation; Microfluorescence

1. Introduction

An increase in cytoplasmic calcium ($[Ca^{2+}]_i$) in pancreatic β-cells is a key step in glucose-induced insulin secretion [1]. In isolated islets of Langerhans, at stimulatory glucose concentrations (above 7 mM) the calcium signal is oscillatory [2]. Such oscillations are originated by the bursting electrical pattern [3–5] that β-cells display when they are recorded within the islet of Langerhans [6]. Each burst of electrical activity [7] or calcium oscillation [8–10] is associated with a pulse of insulin secretion [11,12].

The recording of the electrical activity of β-cells in islets of Langerhans in vivo shows oscillations in the membrane potential [13]. Interestingly, the slope of the electrical response is maximal within the physiological range (5 to 8 mM) of blood glucose concentration. Furthermore, the electrical activity of different cells within the same islet is synchronous [14]. It is therefore important to know whether the islets of Langerhans in vivo also display oscillatory calcium signals and to study its glucose dependence.

In the present study we have used fluorescent indicators combined with image-analysis techniques to monitor $[Ca^{2+}]_i$ in pancreatic islets of Langerhans in vivo. Our approach has proved to be feasible, allowing the characterisation of calcium signals that take place at near-physiological conditions. Such signals seem to originate from oscillatory changes in membrane potential.

2. Materials and methods

Albino mice (8–10 weeks old, 25–35 g weight), bred in our animal house with free access to food and water, were used. Mice were anaesthetised by intraperitoneal injection of 90 mg/kg Nembutal. The degree of anaesthesia was checked periodically during the experiment by exploration of the cutaneous reflexes. The experiments were carried out according to institutional animal care guidelines.

The animal was laparotomised and the duodenal part of the pancreas dissected free from adherence. During the experiment the animal was laid on its back on a heated bed maintained at 37°C. The duodenal part of the pancreas was gently exteriorised on top of a small flat platform covered by a layer of Sylgard and the area close to a superficially located islet immobilised with microdissecting pins. In this way, the preparation was isolated from respiratory and peristaltic movements. The platform also ensured a homogeneous focus level and was attached to a micromanipulator to allow precise positioning [15]. The vena cava and the abdominal aorta were cannulated at their most caudal parts for solution infusion and blood sample collection, respectively. Blood samples (25 μl) from the aorta were analysed for glucose concentration by the glucose oxidase method using a Beckman glucose-analyser-2.

Intracellular calcium was measured by microfluorimetric methods combined with image-analysis techniques. The peritoneum and the capsule of the islet [16] were penetrated with a patch-pipette filled with a solution containing (in mM): NaCl, 140; $CaCl_2$, 2; $MgCl_2$, 1; KCl, 5; HEPES, 20 (pH 7.4); fura-2 AM (100 μM) (Molecular Probes, Eugene OR, USA). Pressure pulses of 1 s duration were delivered over a period of 10 to 15 min, with a 15 s recovery between consecutive pulses. Loading of the extracellular space with the pipette solution could be followed by the transitory swelling of the islet produced by the injection. In parallel experiments, we checked the time course for the clearance of the dye lucifer yellow from the extracellular space of the islet, and found it to be less than 5 s. Before starting the experiment, a period of 30 min was allowed for the complete cleavage of the ester. In general, we found that the indicator was poorly incorporated into the cells. The islets of Langerhans have a substantial cholinergic innervation, making it possible for the cholinesterase activity to interfere with the AM loading of the cells. For this reason we added the acetyl-cholinesterase inhibitor neostigmine (2 μM) to the pipette solution, making the load significantly more efficient. In parallel experiments, we have checked the effect of neostigmine on the electrical activity recorded in vivo, a parameter related to the calcium oscillations (see Section 4), and found that this cholinesterase inhibitor does not have a significant effect. This approach has been successfully used in other preparations to improve loading of the AM form of fluorescent indicators [17].

For excitation of the dye we indirectly used the wavelength pairs 340/380 or 350/380 nm through a water immersion objective (Zeiss Achroplan 40× NA = 0.75) mounted in an upright microscope (Zeiss Axioskop). The fluorescence emitted at 510 nm was recorded by a Hamamatsu C2400 intensifier/Dage 72 video camera [18]. In most experiments, the average of eight images captured at each wavelength was digitised at 8 bits resolution, stored ratioed and spatially analysed using a MCID M4 System, Imaging Res., Ont., Canada.

Excitation of the loaded islets with ultraviolet light at low frequency (one sample/2 min) caused the fluorescence to decrease by half in about 30 min. In later experiments, the anion transport inhibitor probenecid [19] was applied intraperitoneally 5 min before starting to load. Under these conditions the dye retention was significantly

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improved, and after 60 min the islets retained 50% of their initial fluorescence.

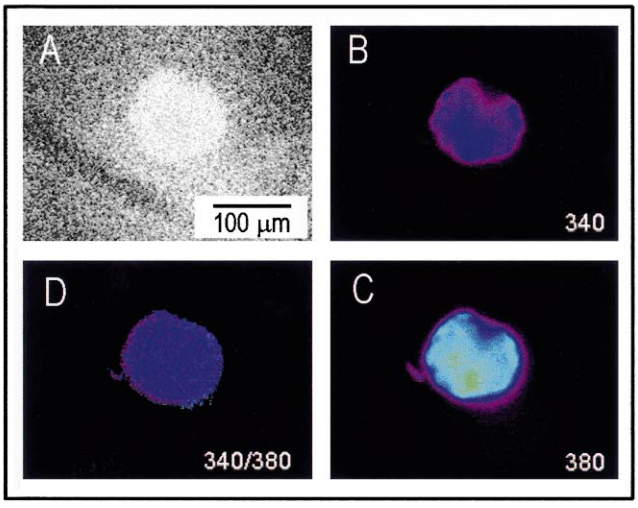
Calibration of the fluorescence signals in terms of $[Ca^{2+}]_i$ using a standard in vitro calibration curve [20] was not possible due to uncertainties about autofluorescence and the behaviour of the indicator inside the cell [21]. Consequently the records are presented as the ratio of the two wavelengths.

3. Results

Fig. 1 (upper panel) shows images of an islet of Langerhans loaded with the calcium indicator fura-2. Fig. 1A shows the islet illuminated with visible light. Fig. 1B,C show false-colour fluorescence images at 340 and 380 nm and Fig. 1D the ratio 340/380. As can be seen, the fluorescence is circumscribed and delimited by the periphery of the islet, with no signs of loading in external zones. In most of the loaded islets, the fluorescence was contained within the islet. Fig. 1B,C show that the fluorescence is not homogeneously distributed within the islet, being maximal at the periphery, mostly in the region close to the injection point (lower left corner in this experiment). The same uneven distribution of fluorescence has been observed in isolated islets [6]. This effect is probably due to restricted diffusion of fura-2 AM. Fig. 1D shows the ratio 340/380 which is relatively homogeneous. The lower panel of Fig. 1 shows fluorescence oscillations recorded in an islet of Langerhans in vivo. The blood glucose concentration measured initially was 7.0 mM. The fluorescence at 340 and 380 nm (middle and lower traces, respectively) shows the relative magnitude of both signals. Despite relatively low levels of fluorescence, the signals at each wavelength show oscillations that are of opposite direction. Thus, at 340 nm each oscillation produces an increase in the fluorescence that is mirrored by a decrease in the fluorescence at 380 nm (indicated by the vertical dotted lines). The opposite direction of the changes proves that the oscillations reflect genuine changes in $[Ca^{2+}]_i$. The upper trace of the figure shows the oscillations in the ratio 340/380, which in this experiment have a frequency of 2.5/min. At blood glucose concentrations in the range 5–10 mM, the mean duration of these oscillations is 21.9 s (S.E.M. ± 6.6, $n = 18$).

Work on isolated islets has shown that electrical activity is synchronous in different cells within the same islet [22,23]. Such synchronicity results in $[Ca^{2+}]_i$ oscillations that occur simultaneously through the islet [24,25]. Furthermore, the simultaneous recording of electrical activity from different cells within the same islet of Langerhans in vivo also shows synchronicity [14].

Fig. 2 shows the analysis of $[Ca^{2+}]_i$ oscillations in different zones of an islet of Langerhans schematically represented in the drawing. The analysed zones are those that show sufficient fluorescence at the lower wavelength. The traces represent the fluorescence ratio 340/380 nm. Every zone oscillates regularly at a frequency close to 2 oscillations/min. Essentially the same synchronic pattern was found in 38 islets from 25 animals. In all islets, no asynchronic patterns were found in any of the regions.



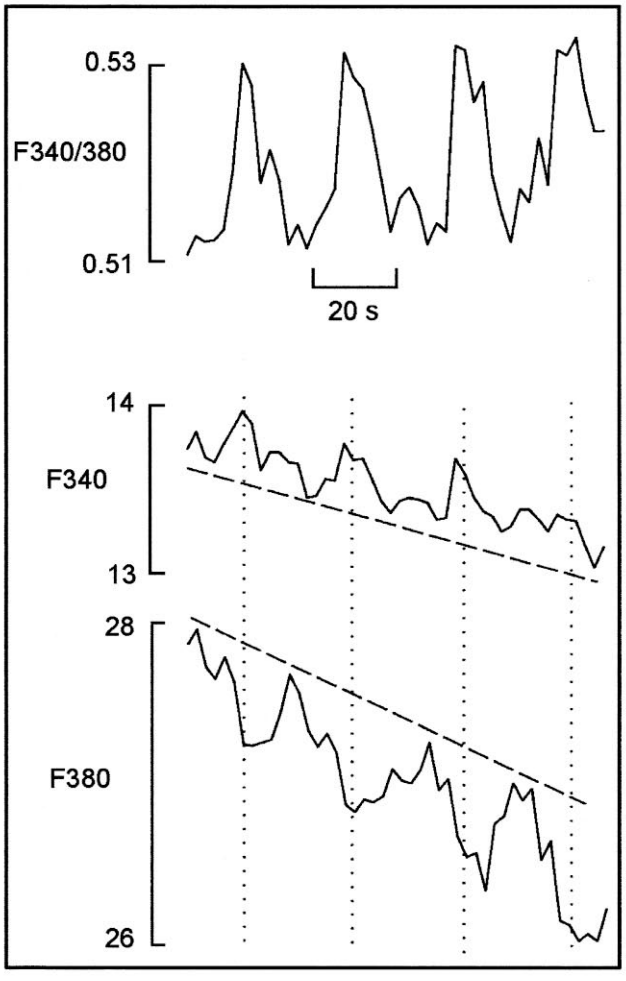


Fig. 1. Fluorescence of an islet of Langerhans in vivo loaded with the calcium indicator fura-2. Upper panel: A: Image of the islet illuminated with visible light. B and C show false-colour images of the islet illuminated at 340 nm (B) and 380 nm (C). Each image is the average of four frames taken at 250 ms intervals. D: False-colour image of the ratio 340/380 nm. Lower panel: the upper trace shows the ratio 340/380 nm and the middle and lower traces the fluorescence at 340 and 380 nm, respectively. The vertical dotted lines illustrate the correspondence of fluorescence changes at both wavelengths. The continuous decrease in fluorescence at each single wavelength (indicated by the dashed line) is mostly due to photobleaching, as the decline in fluorescence is reduced when sampling frequency was decreased (see Section 2).

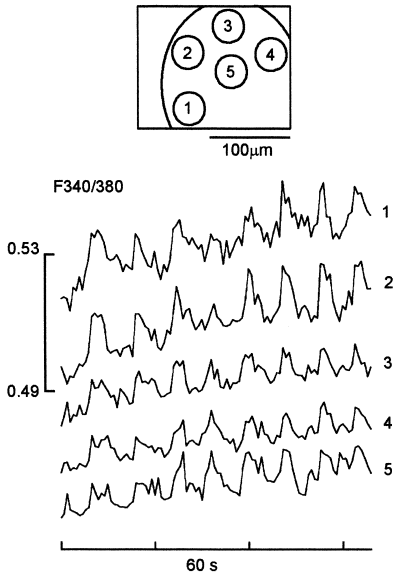


Fig. 2. Spatial analysis of the calcium oscillations in an islet of Langerhans. The zones depicted in the diagram correspond to the zone of the islet with sufficient fluorescence at 340 nm. Consecutive images were taken every 3 s. The same synchronous oscillatory pattern was obtained irrespective of the shape or area analysed.

Fig. 3 shows the effect of increasing blood glucose concentration. The blood glucose concentration at the beginning of recording was 5.15 mM. The different regions of the islet oscillate synchronously. At the point indicated by the arrow, 25 μl of glucose solution (250 mM) was injected as a single bolus into the vena cava. This leads to a transient increase in $[Ca^{2+}]_i$ that lasts for a few minutes, followed by the reappearance of calcium oscillations. The $[Ca^{2+}]_i$ changes induced by

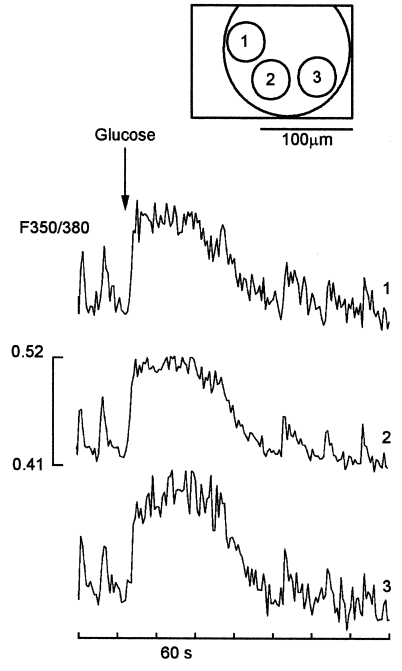


Fig. 3. Effect of increasing blood glucose concentration on $[Ca^{2+}]_i$. At the time indicated by the arrow, 25 μl of saline containing glucose (250 mM) were injected as a single bolus into the vena cava. Other details as in Fig. 2.

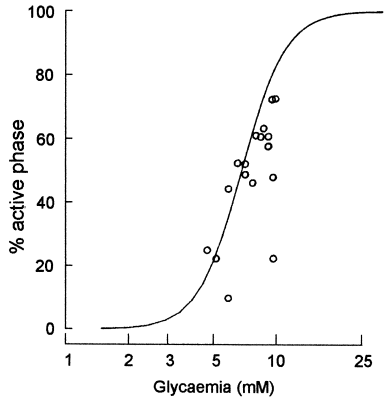


Fig. 4. Relationship between blood glucose concentration and the relative duration of the $[Ca^{2+}]_i$ oscillations. The length of the oscillations was measured as the interval between the half point of the rising and the falling phases, and is expressed as a percentage with respect to the total recording time. The continuous line represents the function describing the relationship between blood glucose concentration and the relative duration of the depolarised phase in membrane potential recorded in vivo (see Fig. 3 in [13]).

the increase in blood glucose occur synchronously throughout the different zones of the islet that could be analysed. Essentially the same synchronic increase in $[Ca^{2+}]_i$, due to glucose injection, was recorded in another 14 islets from nine different animals.

The length of the depolarised phase of the oscillatory electrical activity is directly proportional to the blood glucose concentration both in vitro [3,4] and in vivo [13]. Fig. 4 shows a plot of the relationship between the blood glucose concentration and the relative duration of the $[Ca^{2+}]_i$ oscillations. The duration of each oscillation was measured as the interval between the half rise and half fall of the oscillation. The total time was obtained as the sum of the peaks and troughs of the oscillations. As can be seen, the relative duration of the oscillations augment as blood glucose concentration increases (17 observations from 14 different animals). The continuous line represents the fitting of the duration of the active phase of the electrical activity with respect to blood glucose concentration recorded in vivo (see Fig. 3 in [13]), and has been drawn for comparison with the relative duration of $[Ca^{2+}]_i$ oscillations recorded in vivo.

4. Discussion

The present results show that $[Ca^{2+}]_i$ in islets of Langerhans recorded in vivo, oscillates at physiological blood glucose concentrations. One of the most interesting properties of these oscillations is their synchronicity throughout the islet. Such synchronicity is preserved when the islets are challenged with sudden increases in blood glucose, suggesting that in vivo, the islets of Langerhans are behaving as a functional syncytium.

The characteristics of the $[Ca^{2+}]_i$ oscillations are compatible with having originated from the oscillatory electrical pattern of β-cells. This conclusion is based on the correlation between electrical activity and $[Ca^{2+}]_i$ oscillations found in isolated islets [6,8] and the correlation between blood glucose concentration and electrical activity recorded in vivo [13] and the $[Ca^{2+}]_i$ oscillations described here. The fact that electrical ac-

tivity is synchronous in different β-cells from the same islet of Langerhans in vivo [14] and that $[Ca^{2+}]_i$ oscillations are homogeneous, reinforces such a conclusion. On the other hand, manoeuvres that produce consistent changes in the electrical activity, lead to the expected changes in $[Ca^{2+}]_i$. Thus, an increase in blood glucose leads to a transitory increase in $[Ca^{2+}]_i$. The time course for the rise in $[Ca^{2+}]_i$ is consistent with the time course obtained in electrophysiological recordings in vivo [13]. Conversely, the decrease in blood glucose concentration produced by the administration of insulin to an animal with high blood glucose levels leads to the hyperpolarisation of the membrane potential [26] and the transition in $[Ca^{2+}]_i$ from high levels to oscillations (data not shown). Taken together, these results strongly suggest that $[Ca^{2+}]_i$ oscillations originate from synchronous electrical activity of β-cells within the islet.

In isolated islets, the $[Ca^{2+}]_i$ oscillations lead to discrete pulses of insulin release [8–12]. Therefore, our results permit us to hypothesise that associated with every $[Ca^{2+}]_i$ oscillation observed in vivo, there is a secretory pulse of insulin. If this is the case, the insulin concentration in the islet of Langerhans blood vessels should change at a frequency close to 2 oscillations/min at blood glucose concentrations close to 7 mM. It has been shown that in vivo, the oscillations in the electrical activity of different islets from the same pancreas are not synchronous [14]. Therefore, the pulses in insulin secretion produced by different islets may be out of phase. Work in isolated islets has shown the existence of slow $[Ca^{2+}]_i$ oscillations with periods of several minutes, however, given the limited duration of our recordings we can not say if such oscillations exist in vivo.

In conclusion, our results indicate that the use of conventional microfluorescence techniques allows the monitoring of $[Ca^{2+}]_i$ dynamics in the endocrine pancreas of living animals. At physiological blood glucose concentrations, the $[Ca^{2+}]_i$ signal in islets of Langerhans is oscillatory and synchronous throughout the islet. Furthermore, the length and frequency of the $[Ca^{2+}]_i$ signals, as well as its glucose-dependence, are compatible with having originated from the electrical activity.

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