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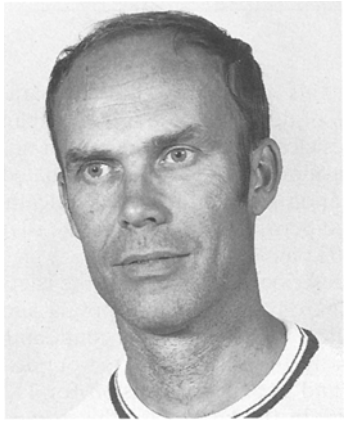
Bo Hellman
Minkowski Award, 1969, Montpellier



Bo Hellman was born in 1930 in Mariestad, Sweden. In 1950 he started medical studies in Uppsala and became a licensed physician in 1958. In the following year he completed a research degree (M.D.) and was appointed Assistant Professor at the Department of Histology, University of Uppsala. In 1963 he got a research position in experimental diabetes from the Swedish Medical Research Council, and 1964–1966 he was Associate Professor at Karolinska Institute, Stockholm. In 1966 he moved to the University of Umeå as a Professor of Histology. Since 1976 he has been Professor at the Department of Medical Cell Biology, University of Uppsala. Since 1953 he has published 358 papers, mainly dealing with pancreatic islets. Bo Hellman has been part of the organizing committee for two international islet symposia, one held in Uppsala in 1963 and the other in 1973 in Umeå for the centennial of Paul Langerhans' discovery of the islets. His activities in the European Association for the Study of Diabetes have included positions as Vice President 1970–1973 and Honorary Secretary 1973–1976. Since 1992 he has belonged to the Life Science panel at the Commission of the European Community. In 1983 he was awarded the Eriksson prize from the Royal Swedish Academy of Sciences.

Glucose induces oscillatory Ca^{2+} signalling and insulin release in human pancreatic beta cells

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Summary Mechanisms of pulsatile insulin release in man were explored by studying the induction of oscillatory Ca^{2+} signals in individual beta cells and islets isolated from the human pancreas. Evidence was provided for a glucose-induced closure of ATP-regulated K^+ channels, resulting in voltage-dependent entry of Ca^{2+}. The observation of step-wise increases of capacitance in response to depolarizing pulses suggests that an enhanced influx of Ca^{2+} is an effective means of stimulating the secretory activity of the isolated human beta cell. Activation of muscarinic receptors (1–10 μmol/l carbachol) and of purinergic P_2 receptors (0.01–1 μmol/l ATP) resulted in repetitive transients followed by sustained elevation of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$). Periodic mobilisation of intracellular calcium was seen also when injecting 100 μmol/l GTP-γ-S into beta cells hyperpolarized to –70 mV. Individual beta cells responded to glucose and tolbutamide with increases of $[Ca^{2+}]_i$, manifested either as large amplitude oscillations (frequency 0.1–0.5/min) or as a sustained elevation. Glucose regulation was based on sudden transitions between the basal and the two alternative states of raised $[Ca^{2+}]_i$ at threshold concentrations of the su-

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gar characteristic for the individual beta cells. The oscillatory characteristics of coupled cells were determined collectively rather than by particular pacemaker cells. In intact pancreatic islets the glucose induction of well-synchronized $[Ca^{2+}]_i$ oscillations had its counterpart in 2–5 min pulses of insulin. Each of these pulses could be resolved into regularly occurring short insulin transients. It is concluded that glucose stimulation of insulin release in man is determined by the number of beta cells entering into a state with Ca^{2+}-induced secretory pulses. [Diabetologia (1994) 37 [Suppl 2]: S11–S20]

Key words Ca^{2+} signalling, diabetes mellitus, glucose, insulin secretion, islets of Langerhans, oscillations, pancreatic beta cells.

Although studies of the calcium handling of pancreatic beta cells have been performed essentially in experimental animals there are reasons for believing that Ca^{2+} also has a key role in the insulin secretory process in man [1]. Substances known to promote the entry of Ca^{2+} by depolarizing the beta cells, such as glucose, sulphonylureas and arginine, increase the levels of circulating insulin in normal individuals, whereas the hyperpolarizing diazoxide has the opposite effect [2]. An important feature of circulating insulin is its cyclic variations, reflecting pulsatile release of the hormone from the pancreas [3]. Taking 1-min samples of venous blood it has been possible to demonstrate 10–14 min cycles of insulin, which cannot be accounted for by variations of the glucose concentration [3–5]. Evidence has been provided to show that loss of the regular insulin oscillations is a very early phenomenon in the development of both insulin [6] and non-insulin-dependent diabetes mellitus [7].

An important step in the understanding of pulsatile insulin release was the discovery that glucose induces periodic changes of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated beta cells from mice and rats [8, 9]. It will now be shown that the human pancreatic beta cells also respond to glucose with oscillatory $[Ca^{2+}]_i$ associated with a pulsatile insulin release. Evidence will be provided that the periodic variation of circulating insulin is accounted for by a co-ordination of $[Ca^{2+}]_i$ cycles originating in the individual beta cells and that the total secretory activity is determined by the number of cells recruited into active phases.

Subjects and methods

Isolation of islets and preparation of beta cells. Pancreatic glands were excised from eight human cadaveric organ donors and transported to the Central Unit for beta cell transplantation (Medical Campus, Vrije Universitet Brussels, Brussels, Belgium). The mean age ± SEM of the donors was 39±5 years. The islets were isolated with a procedure involving ductal distension with collagenase and Ficoll gradient purification [10]. The isolated islets were cultured for 3–7 days in Ham's F 10 medium containing 6.1 mmol/l glucose and supplemented with 0.5 % bovine serum albumin, 0.08 mg/ml penicillin and 0.1 mg/ml streptomycin. After this culture period the preparations contained less than 10 % of dead cells and also less than 10 % of cells with exocrine vesicles as judged by electron microscopy. The prevalence of insulin-positive cells, as evaluated by light microscopic examination of immunocytochemically stained sections of islets, varied between 14–72 % and the islet content of insulin was 0.17±0.02 pmol/ng DNA.

Adhering to a previously described protocol [11], the islets were subsequently sent by air from Brussels to Uppsala and stored for 1–3 days in RPMI 1640 medium containing 5.6 mmol/l glucose. Alternatively, the cultured islets were dispersed into single cells [12] and kept in suspension for 1–2 days before the transportation. In the latter case the storage in RPMI 1640 medium was associated with attachment of the cells to circular 25-mm diameter cover glasses. When not otherwise stated both the experiments with intact islets and isolated cells were performed at 37 °C with a basal medium containing (mmol/l): NaCl 125, KCl 5.6, $CaCl_2$ 2.6, $MgCl_2$ 1.2 and HEPES 25 titrated to pH 7.4 with NaOH. The medium was perifused at a rate of 0.14–1.0 ml/min with the cells or islets attached to cover glasses used as the bottom of open chambers placed on the stage of an inverted microscope (patch clamp analyses and $[Ca^{2+}]_i$ measurements) or with single islets introduced into a closed 10-µl chamber (insulin release). Isolated cells, which responded appropriately to tolbutamide or diazoxide, were classified as beta cells.

Patch clamp analyses. Standard patch clamp techniques [13] were employed for measuring single channels in the cell-attached configuration and making intracellular perfusions in the whole cell configuration. Membrane capacitance was measured by the perforated patch approach using a 2-phase software lock-in amplifier adding a 50 mV peak-to-peak 800 Hz sine wave to the command potential.

Measurements of cytoplasmic Ca^{2+}. The indicators fura-2 and indo-1 were employed for measuring $[Ca^{2+}]_i$ by dual wavelength fluorometry following the principles of Grynkiewicz et al. [14]. The emitted fluorescence was recorded with a photomultiplier [8, 15] or by an intensified video camera [16] as previously described. When not injected with a patch clamp pipette the indicators were introduced into the cells during 30–40 min incubation with their acetoxymethyl esters at concentrations of 1 (cells attached to cover glasses) or 2 µmol/l (free floating islets). Before measuring $[Ca^{2+}]_i$ the islets loaded with fura-2 were placed on the central part of a cover glass coated with poly-L-lysine.

Measurements of insulin release. Perifusate from individual islets was collected in 2.5–20 s fractions and immediately cooled by ice. Insulin was measured as previously described [17] using competitive ELISA with the insulin antibody immobilized directly onto the solid phase. Amounts of insulin down to 50

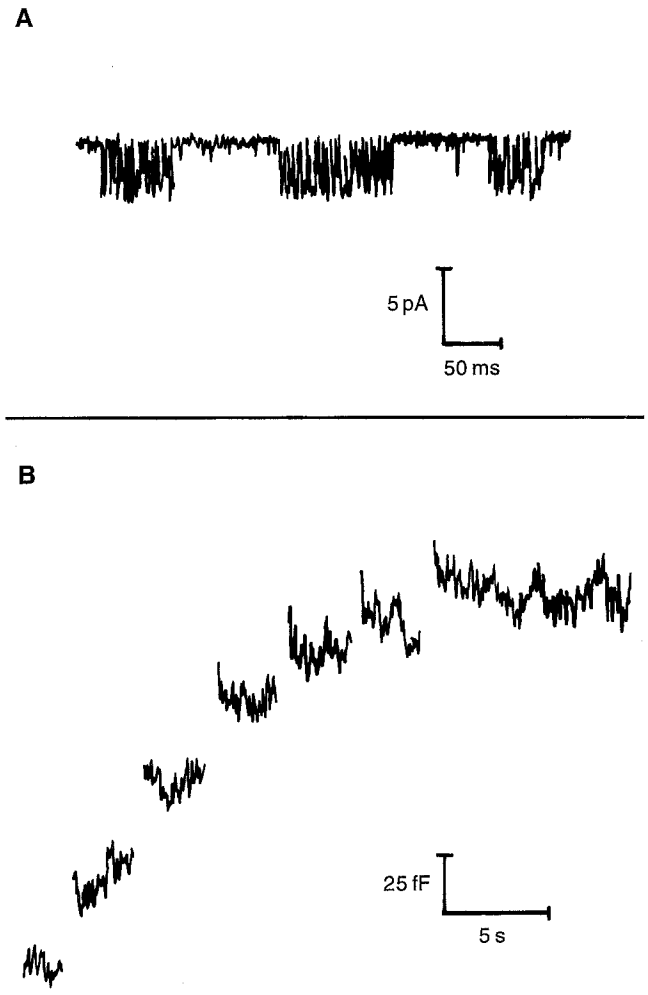

Fig. 1 A, B. Patch clamp analyses of human beta cells. Panel A: ATP-regulated K^+ channel activity observed in the cell-attached configuration with a high K^+ (140 mmol/l) in the pipette. Panel B: Capacitance increases evoked by 500-ms step depolarisations from –70 to 0 mV at 32 °C

amol were calculated from linear standard curves in semilogarithmic plots with correlation coefficients of 0.93–0.98. The inter- and intraassay variations were always less than 10 %. The rate of insulin release was normalized to dry weight after freeze-drying and weighing the islets on a quartz fiber balance.

Results

Stimulus-secretion coupling of individual beta cells. The beta cells were equipped with K^+ channels with features characteristic of the ATP-regulated channels known to determine the resting membrane potential (Fig. 1A). These channels (conductance 67 pS) were closed when the glucose was raised above 5 mmol/l and opened in the presence of 400 μmol/l diazoxide. Combining capacitance measurements with the perforated patch approach it was found that depolarizing pulses initiated steps of capacitance increase. In the experiment shown in Figure 1B there was a capacitance increase of 40–50 fF

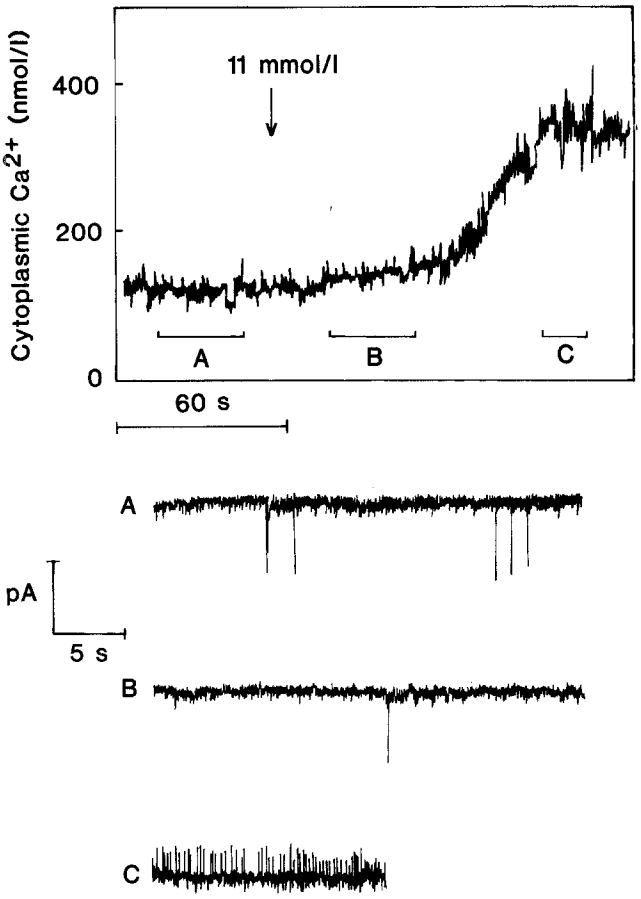

Fig. 2. Effects of raising glucose from 3 to 11 mmol/l on the cytoplasmic Ca^{2+} concentration (upper panel) and the electrical activity during periods A-C (lower panel) at 35 °C. The electrical activity was measured in the cell-attached configuration with a high K^+ (140 mmol/l) in the patch pipette

(= release of 20–25 granules) in response to 500 ms pulses of depolarisation from –70 to 0 mV. Further evidence for a glucose-induced entry of Ca^{2+} mediated by the closure of ATP-regulated K^+ channels is given in Figure 2. It can be seen how increasing glucose from 3 to 11 mmol/l results in a rise of $[Ca^{2+}]_i$ associated with the closure of the ATP-regulated K^+ channels and the appearance of action potentials.

Cyclic mobilisation of intracellular calcium. The question of whether inositol-1,4,5-trisphosphate induces oscillations by mobilising intracellular Ca^{2+} was approached by activating receptors coupled via G proteins to phospholipid breakdown. The experiments were performed under conditions known to favour the uptake of Ca^{2+} into the endoplasmic reticulum (20 mmol/l glucose) and minimize its entry via potential-dependent channels (10–50 μmol/l methoxyverapamil). Both the activation of muscarinic receptors with carbachol and of purinergic P_2 receptors with ATP resulted in a prompt response with one or more $[Ca^{2+}]_i$ peaks followed by a sustained increase (Fig. 3). With removal of the agonists $[Ca^{2+}]_i$ dropped

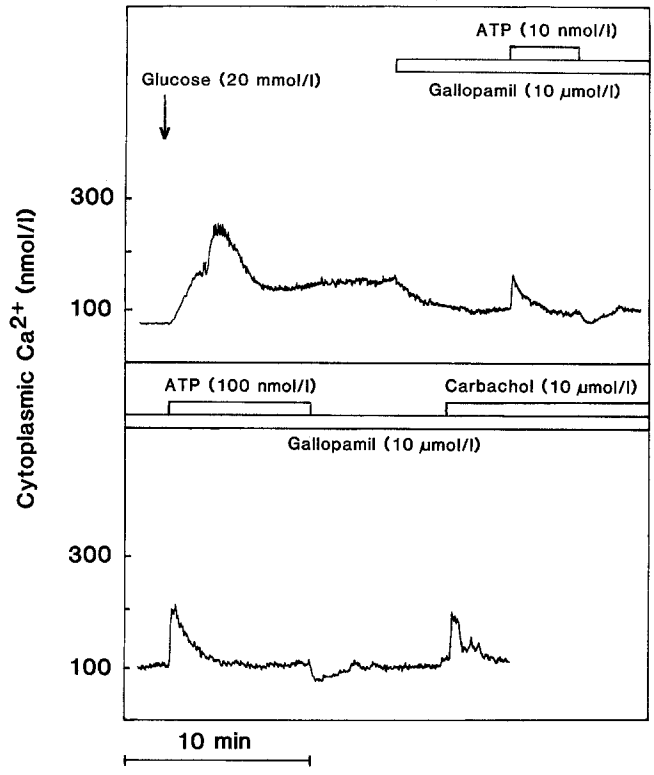

Fig. 3. Effects of ATP and carbachol on cytoplasmic Ca^{2+} in a human beta cell exposed to 20 mmol/l glucose in the presence of methoxy verapamil

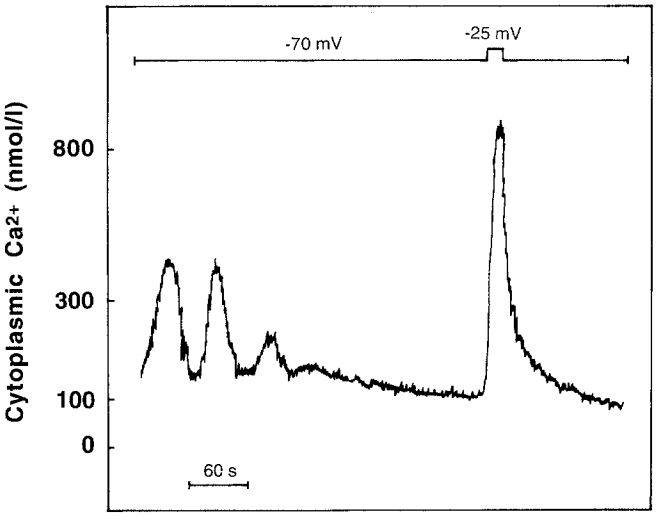

Fig. 4. Cyclic mobilisation of intracellular Ca^{2+} in a human beta cell perfused internally with a medium containing 100 μmol/l GTP-γ-S, 3 mmol/l ATP and 100 μmol/l indo-1 at a membrane potential of either –70 or –25 mV. The experiments were performed at room temperature using the whole cell configuration of the patch clamp technique

below the initial level. The beta cells were very sensitive to the ATP challenge, a prominent $[Ca^{2+}]_i$ peak already being seen at a concentration of 10 nmol/l. It was tested whether it was possible to induce a periodic release of Ca^{2+} from intracellular stores also by infusing the non-hydrolysable GTP analogue GTP-γ-S into hyperpolarized beta cells. This approach resulted in a few oscillations with a frequency of about 1 per min (Fig. 4).

Glucose induction of cytoplasmic Ca^{2+} oscillations. The beta cells often responded to an increase of glucose by oscillations of $[Ca^{2+}]_i$. In addition to large amplitude oscillations from the basal level with a frequency of 0.1–0.5 per min (type-a oscillations), there was also a smaller variant (type-b oscillations) superimposed on an elevated $[Ca^{2+}]_i$ (Fig. 5A). The induction of type-a oscillations was not specific for glucose but also resulted from tolbutamide (Fig. 5B). The proportion of beta cells reacting with type-a oscillations decreased when extending exposure to ultraviolet-light beyond 30 min. Glucose was a determinant for the transition from the basal $[Ca^{2+}]_i$ to type-a oscillations at threshold concentrations characteristic of the individual beta cell. However, the shapes and frequencies of these oscillations remained essentially unaffected when raising further the concentration of the sugar. It was possible to transform the oscillatory state into a steady-state increase by addition of 10 nmol/l glucagon (Fig. 6A) or 10 mmol/l glycine (not shown). Figure 6B illustrates a partial transformation with as little as 1 mmol/l glycine.

Using digital image analyses it was found that the oscillatory Ca^{2+} signal propagates between adjacent cells. The result of such an analysis is shown in Figure 7. All cells in the cluster (labelled A-G) had a well-synchronized $[Ca^{2+}]_i$ response to the tolbutamide challenge and a subsequent increase of glucose from 3 to 20 mmol/l. Since consecutive oscillations could originate from different parts of a cluster, there was no evidence for particular pacemaker cells.

Ca^{2+} signalling and insulin release in intact islets. Glucose induced $[Ca^{2+}]_i$ oscillations also in intact pancreatic islets. A characteristic response pattern is shown in Figure 8. It can be seen how a rise of glucose from 3 to 20 mmol/l results in an increase of $[Ca^{2+}]_i$ followed by the generation of slow sinusoidal oscillations from an elevated level. The periods of these oscillations were 2–5 min. Although displaying considerable regional differences with regard to amplitudes, the oscillations were well synchronized throughout the islet.

Individual human islets responded to glucose with periodic release of insulin. Figure 9 shows the results of measuring insulin in samples of perifusate taken at 20-s intervals. The left panels indicate the oscillatory behaviour in the presence of 11 mmol/l glucose before (panel A) and after (panel C) reduction of the assay noise by plotting a three-point moving average. The right panels show the corresponding results when the same islet was exposed to 20 mmol/l glucose. The increase of the secretory rate obtained

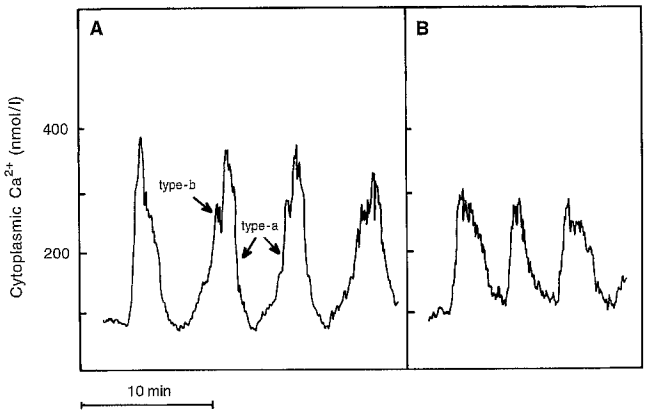

Fig. 5 A, B. Cytoplasmic Ca^{2+} oscillations in human beta cells induced by 20 mmol/l glucose (panel A) or 1 mmol/l tolbutamide (panel B) added to a medium containing 3 mmol/l glucose

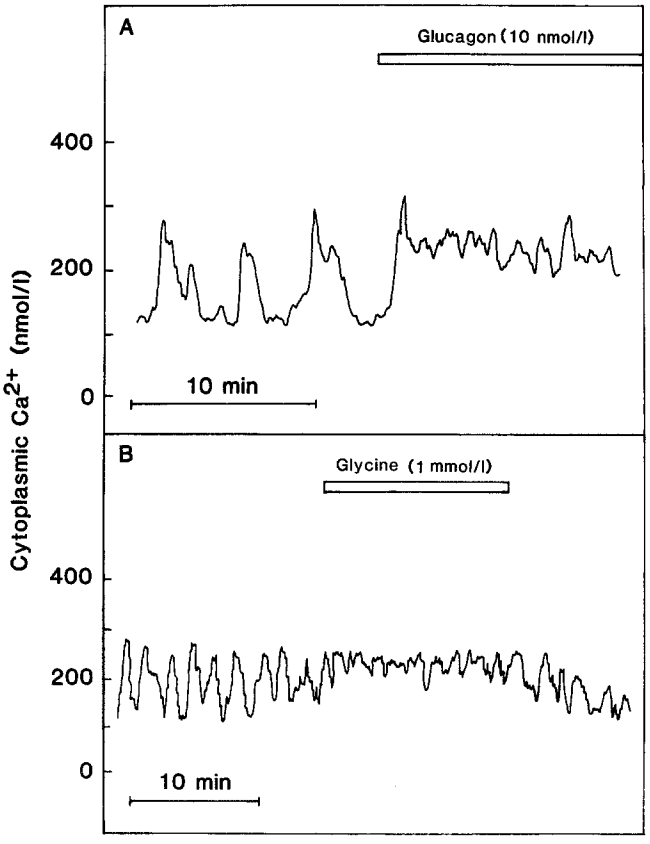

Fig. 6 A, B. Effects of glucagon (panel A) and glycine (panel B) on cytoplasmic Ca^{2+} oscillations induced by 20 mmol/l glucose. The observations in panel A refer to an isolated beta cell and those in panel B to a cluster of eight human islet cells

when raising the glucose concentration was manifested in higher amplitudes of the pulses leaving their frequencies unaffected. The pulse period obtained in five islet donors was 3.69±0.45 min (mean values ± SEM).

Taking advantage of the largest islets isolated (dry weight 3 μg) it was possible to make provisional measurements of insulin in perifusate taken at intervals as short as 2.5 s. With the improved time resolution the previously described 2–5 min pulses could be resolved into regularly occurring short (< 15 s) transients (Fig. 10).

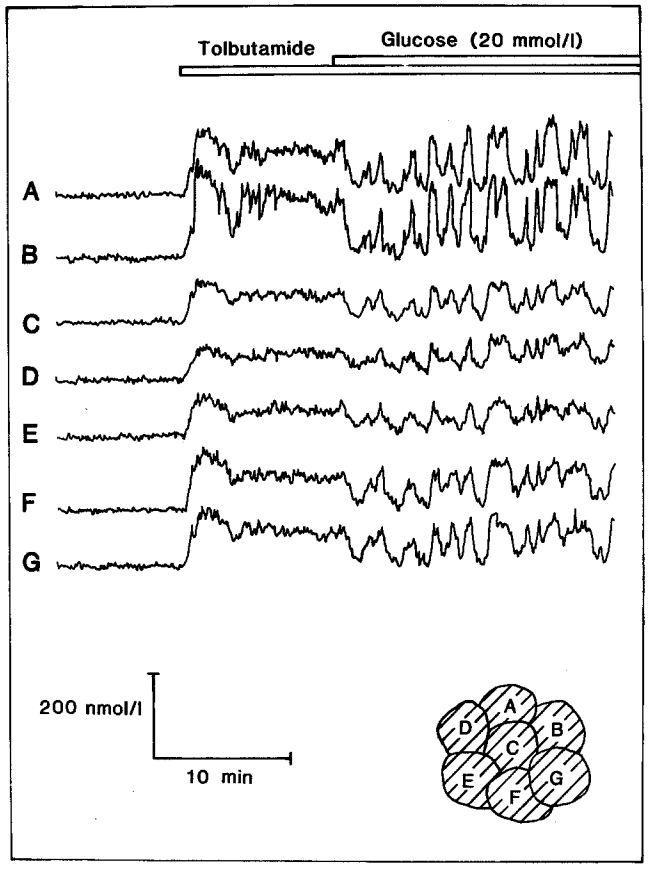

Fig. 7. Synchronisation of the cytoplasmic Ca^{2+} signal in a cluster of seven human islet cells. After perifusion with 3 mmol/l glucose, tolbutamide was added at a concentration of 1 mmol/l and glucose raised to 20 mmol/l as indicated by the horizontal bars. The traces show $[Ca^{2+}]_i$ for the cells marked A-G

Discussion

The possibility of observing pulsatile Ca^{2+} signalling in pancreatic beta cells is specifically dependent on the methodology employed. With improved procedures for cell isolation and efforts to minimize the exposure to ultraviolet light during the measurements with the fura-2 indicator there has been a substantial increase in the percentage of beta cells responding with $[Ca^{2+}]_i$ oscillations. After 5 years experience we find that the proportion of mouse beta cells responding to 11 mmol/l glucose with $[Ca^{2+}]_i$ oscillations is as high as 85 % [18]. It is important to maintain an adequate oxygen supply during the isolation of the islets used for preparing the individual beta cells. This requirement is most easily satisfied in species such as mice and rats with their thin pancreas. However, as

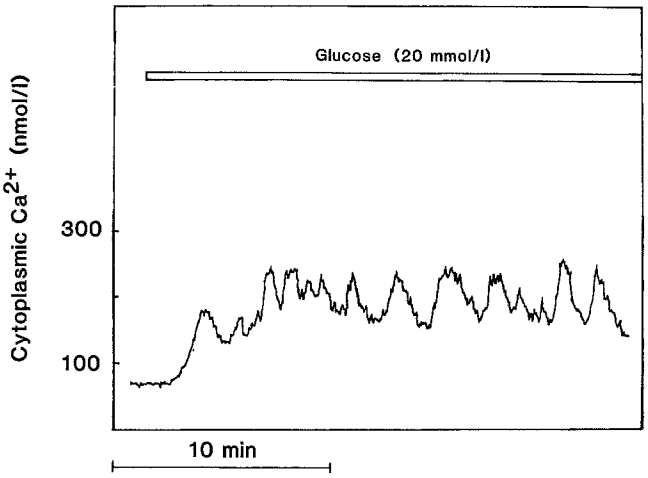

Fig. 8. Glucose-induced responses of cytoplasmic Ca^{2+} in a human islet. The concentration of glucose was raised from 3 to 20 mmol/l during the period indicated by the horizontal bar

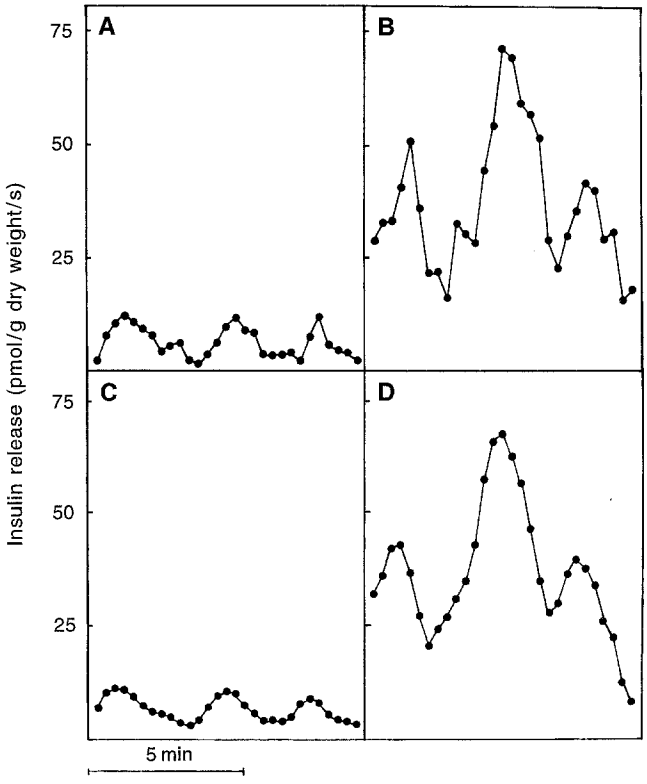

Fig. 9 A–D. Insulin release from a human islet exposed to 11 (left panels) and 20 (right panels) mmol/l glucose. Panels A and B show the secretory rates after 10 min of exposure to each of the glucose concentrations with a perifusion rate of 180 µl/min and a sampling period of 20 s. Panels C and D show the results of plotting the three-point moving averages to reduce the assay noise

shown in the present study, it has now been possible to demonstrate $[Ca^{2+}]_i$ oscillations also in beta cells isolated from the solid human pancreas, indicating that this phenomenon is not restricted to experimental animals.

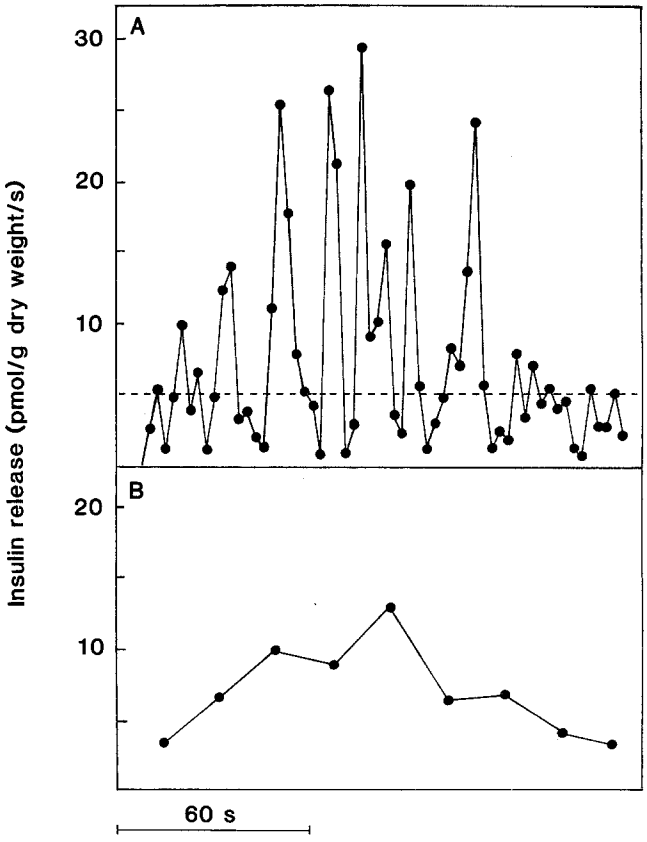

Fig. 10 A, B. Insulin release from a human islet exposed to 11 mmol/l glucose. Panel A shows the rate of secretion obtained with a sampling time of 2.5 s, and panel B the rate recalculated with a sampling time of 17.5 s (i.e. the average of seven 2.5 s periods). The perifusion rate was 140 µl/min. Secretory rates below the dotted line should be regarded as provisional in view of a non-linearity of the insulin standard curve for amounts below 50 amol

Previous studies have indicated that external ATP induces glucose-dependent mobilisation of intracellular calcium in mouse islets similar to that obtained when polyphosphoinositide breakdown is activated via muscarinic receptors [19, 20]. It was evident that this mechanism also operates in human beta cells. Whereas receptor-mediated generation of inositol-1,4,5-trisphosphate is known to generate sustained oscillations in various types of cells [21], the human beta cells responded to ATP and the muscarinic agonist carbachol with one or a few transients superimposed on an elevated $[Ca^{2+}]_i$. Neither was it possible to get more than a couple of oscillations by direct activation of G-proteins using a protocol effective in inducing sustained oscillations in mouse beta cells [15]. The demonstration of a clear mobilisation of intracellular calcium with as little as 10 nmol/l ATP raises the question of a physiological role for the P_2-purinoceptors in insulin release in man. Whereas little ATP can be detected in the circulation, release from nerve endings may result in the accumulation of the nucleotide in the immediate vicinity of the beta cells. An-

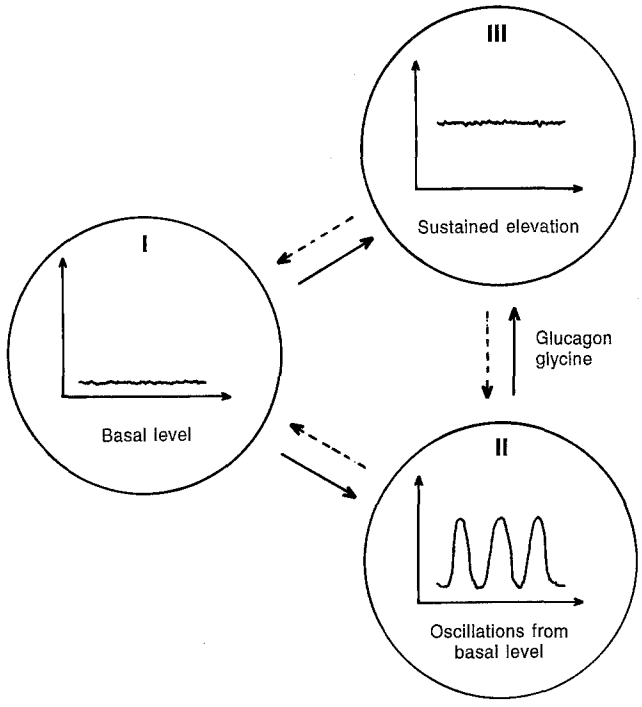

Fig. 11. Schematic illustration of how glucose transforms basal cytoplasmic Ca^{2+} (state I) in human beta cells into oscillations (state II) or sustained elevation (state III). The transitions between the different states are reversible events (dotted lines), which appear suddenly at threshold concentrations of glucose characteristic for the individual beta cell. Glucagon and glycine are examples of compounds which promote transformation of the cytoplasmic Ca^{2+} oscillations into sustained increase

other and probably more important source of ATP and ADP is the beta cells themselves. Taking into account that insulin secretory granules have been reported [22] to contain more than 100-fold the concentration of ATP required for raising $[Ca^{2+}]_i$ in the human beta cell, it seems likely that the P_2-purinoceptor is part of a system for amplifying the secretory response to glucose and other stimulators of insulin release.

It is supposed that glucose-induced insulin release is due to the closure of an ATP-regulated K^+ channel with a resulting depolarisation promoting voltage-dependent Ca^{2+} entry [1]. The present observations confirm previous reports [23, 24] that such K^+ channels also exist in human pancreatic beta cells. Moreover, it was shown that a glucose-induced depolarisation of the beta cells results in increase of $[Ca^{2+}]_i$ associated with the appearance of action potentials. A step-wise increase of the cell capacitance is a useful indicator of exocytosis, reflecting incorporation of granule vesicle membranes into the plasma membrane [25]. Using this approach it was found that an enhanced influx of Ca^{2+} is an effective means of stimulating the secretory activity of the isolated human beta cell. The demonstration of distinct steps of capacitance increases in response to depolarizing pulses is in accordance with recent observations [24]. Studies of rodent islets have revealed that glucose-induced entry of Ca^{2+} is a cyclic process, resulting in the generation of slow type-a and fast type-b oscillations of $[Ca^{2+}]_i$ [9, 18]. It seems likely from our observation of similar types of oscillations that glucose induces periodic entry of Ca^{2+} also in human beta cells. It is important to note that this rhythmic behaviour is an intrinsic property of these cells and not the result of neural pacemaker activity.

It was possible to demonstrate three states in the action of glucose on $[Ca^{2+}]_i$ in the individual human beta cell (Fig. 11). Increase of $[Ca^{2+}]_i$ from the basal level was manifested either as large amplitude oscillations or as a sustained elevation. There are reasons for believing that the normal reaction of the healthy beta cell to increases of glucose is a transition to the oscillatory state. Studies of rodent beta cells have indicated that minor damage by toxic agents [26] or ultraviolet light [27] modifies the response towards a sustained elevation. In the present study the $[Ca^{2+}]_i$ oscillations tended to disappear with prolongation of the exposure to ultraviolet light during the measurements with the fura-2 indicator. A pertinent question is how the oscillatory pattern of $[Ca^{2+}]_i$ is related to the ability of the beta cells to recognize the glucose stimulus. In view of the ischaemic problems inherent in the procurement of the human islets (see above) and the limited amounts of such material available, it has not been possible to analyse this problem systematically. Nevertheless, the present data provide strong support for the view that, in analogy to the situation in mice and rats [9, 18], the action of glucose on the time-averaged $[Ca^{2+}]_i$ is determined by sudden transitions between oscillatory and steady-state levels and not by modifications of the shapes or frequencies of the oscillations. By responding to these transitions at different threshold concentrations of glucose, the individual beta cell behaves as predicted from the observations of a heterogeneity among the beta-cell population [28].

Additional evidence for similarities between the beta cells from mice and humans was obtained by the demonstration that glucagon and glycine transform oscillatory $[Ca^{2+}]_i$ into a sustained elevation. The observed glucagon effect is consistent with a role for cyclic AMP both in promoting the appearance of glucose-induced $[Ca^{2+}]_i$ oscillations and transforming existing ones into sustained increase [29]. The fact that the poorly metabolized glycine induced a similar transformation of the type-a oscillations might depend on its co-transport with Na^+ [30]. In the light of observations indicating that increase of the Na^+ permeability perturbs the delicate balance between the influx and removal of Ca^{2+} [18], it is not surprising that the beta cells must be in a satisfactory condition to react with $[Ca^{2+}]_i$ oscillations in response to glucose stimulation.

Table 1. Pulse periods of insulin release at different organizational levels of human beta cells

Organization level	Pulse period (min)
Individual beta cells	2–10[a]
Isolated islets	2–5
Isolated pancreas	6[b]
Pancreas in situ	10–14[c]

[a] Data for individual beta cells refer to those observed for large amplitude oscillations of cytoplasmic Ca^{2+}.
[b] Data for the isolated pancreas are taken from [37]. [c] Data for in situ pancreas reflect the cycles of plasma insulin known to occur in healthy humans [3–5]

When analysing the mechanisms for pulsatile insulin release it is essential to know how the beta cells interact with regard to the generation and propagation of the Ca^{2+} signals. In our approach to this problem clusters of cells were loaded with the fura-2 indicator and taken for digital image analysis during exposure to elevated concentrations of glucose. It was shown that the cells are functionally coupled, resulting in a synchronization of the oscillatory Ca^{2+} signals. In accordance with previous observations in mice [16], the oscillatory characteristics were determined collectively rather than by particular pacemaker cells. Extending the measurements to whole islets, the response to glucose was found to be manifested as an elevated $[Ca^{2+}]_i$ often associated with superimposed slow oscillations. Similar slow oscillations have been observed in mouse islets and regarded as reflecting the synchronization of the type-a oscillations seen in individual beta cells [18]. In a recent report [31] a human islet was found to react to glucose with irregularly shaped $[Ca^{2+}]_i$ oscillations. It is unclear whether these oscillations (duration 70–80 s) are equivalent to those observed in the present study or reflect a variety of the fast oscillations commonly seen in mouse islets [32, 33].

It has been reported that isolated rodent islets respond to glucose with pulsatile insulin release [3]. In the mouse the oscillatory islet Ca^{2+} is supposed to account for a pulsatile insulin release not only because of the established role of Ca^{2+} in initiating secretion, but also because the insulin pulses appear in synchrony with the slow $[Ca^{2+}]_i$ oscillations [18]. When samples of the perifusate from five human insulinomas were analysed at intervals of 3.2 min, insulin was found to be released in periods of 28 min [34]. Using considerably shorter sampling periods we have now been able to demonstrate that human islets release insulin with a frequency similar to the slow islet oscillations of $[Ca^{2+}]_i$. In accordance with observations made with isolated mouse islets [17], glucose regulation of the secretory activity was manifested as alterations of the amplitudes of the insulin pulses leaving their frequencies unaffected. The existence of such a reaction pattern is consistent with previous observations of how glucose affects oscillating plasma insulin [35], supporting the notion that also in man the effect of the sugar is to recruit beta cells into the secretory phase.

It was recently discovered that the sinusoidal insulin pulses generated in the glucose-stimulated mouse islet can be resolved into regularly occurring fast transients [18, 36]. The present study reveals that this is the case also for human islets. Future analyses of the details of insulin release from individual human beta cells will throw more light on these eruptions of secretory activity. It has been proposed that the fast transients of insulin release in experimental animals may reflect the presence of rapid oscillations of $[Ca^{2+}]_i$ [18].

The present knowledge of how the pulses of insulin release are affected by the organization of the human beta cells is summarized in Table 1. Although as yet it has not been measured directly, it can be postulated that individual beta cells release insulin at periods equivalent to the type-a oscillations of $[Ca^{2+}]_i$. Accordingly, when present within an islet removed from the pancreas, the beta cells will generate synchronized insulin pulses with a duration similar to the shortest ones in individual cells. During in vitro perfusion of the human pancreas the pulse period is 6 min [37], which is half as long as that observed for circulating insulin [3–5]. A prolongation of the insulin release pulses mediated by intra- and extrapancreatic factors is not unique for humans. There are similar differences between the in vitro and the in vivo situation in rhesus monkeys [38]. Moreover, the pulses of insulin release observed with isolated rat islets [17] are shorter than the 5–6 min periods recorded during in vitro perfusion of the pancreas itself [39, 40].

An oscillatory rise of $[Ca^{2+}]_i$ may have several advantages compared to a steady-state increase. In generating pulsatile secretory activity the $[Ca^{2+}]_i$ oscillations provide the fluctuations of circulating insulin supposed to prevent the down regulation of the peripheral receptors. In support of this idea the recycling time for the receptors has been found to be shorter than the periods for the plasma insulin pulses [41]. The importance of the pulse frequency for the insulin effect is emphasized from the observation that periods of 13 but not of 26 min induce greater inhibition of the hepatic glucose production than does continous delivery of the hormone [42]. Another advantage of an oscillatory Ca^{2+} signal may be to prevent desensitization of the secretory machinery of the beta cells. Studies of permeabilized beta cells have revealed that prolonged exposure to a high concentration of Ca^{2+} makes the secretory machinery refractory to the Ca^{2+} signal [43]. It is possible that the loss of the regular cycles of plasma insulin during the development of human diabetes [6, 7] reflects a sustained rise of $[Ca^{2+}]_i$ in the stimulated beta cells.

Ca^{2+} is known to activate various autolytic processes by stimulating phospholipases, proteases and endonucleases [44]. Sustained rise of $[Ca^{2+}]_i$ may therefore induce a vicious circle, aggravating a primary lesion of the diabetic beta cell.

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