Dynamics of Calcium-Induced Insulin Release

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Summary. Extracellular Ca^{2+} , at concentrations exceeding 10 mmol/l, causes a dose-related stimulation of insulin release. The dynamics of Ca^{2+} -induced insulin release are characterized by a quick onset, a progressive build-up and a later return towards basal secretory rate. The release of insulin evoked by Ca^{2+} is inhibited in the presence of either Mg^{2+} (10 mmol/l) or the organic Ca^{2+} -antagonist verapamil (81 $\mu\text{mol/l}$), both of which are known to inhibit Ca^{2+} entry in the B-cell. Glucose and theophylline, which are thought to affect the net uptake or intracellular distribution of Ca^{2+} in the B-cell, both enhance Ca^{2+} -induced insulin release. As little as 2.7 mmol/l glucose is sufficient to augment Ca^{2+} -induced insulin secretion. Exposure of the pancreas to somatostatin significantly retards the secretory response to Ca^{2+} . Cytochalasin B potentiates the insulin release evoked by Ca^{2+} (12 mmol/l) and lowers the threshold concentration of Ca^{2+} required to stimulate secretion. These data suggest that high extracellular Ca^{2+} concentrations may sufficiently increase the amount of Ca^{2+} accumulated in the B-cell to eventually trigger insulin release. Agents known to cause a remodelling of Ca^{2+} fluxes across membrane systems in the B-cell interfere with Ca^{2+} -induced insulin release, a process also dependent on the integrity of the cytochalasin B-sensitive microfilamentous effector system.

Key words: Ca^{2+} , insulin release, Mg^{2+} , verapamil, somatostatin, cytochalasin B, glucose, theophylline, perfused pancreas.

Intracellular Ca^{2+} plays an essential rôle in the process of insulin release [1]. It is generally considered that Ca^{2+} acts as a trigger for the machinery controlling the exocytosis of secretory granules, whatever agent is used to stimulate insulin release [2]. This concept is supported by the recent finding that

Ca^{2+} itself is able to promote insulin release [3, 4]. The secretory response evoked by high extracellular Ca^{2+} concentrations is influenced by agents known to interfere with Ca^{2+} fluxes into or within the B-cell, such as verapamil, L-epinephrine and methylxanthines [3, 4].

The present experiments were designed to characterize the dynamics and mechanism of Ca^{2+} -induced insulin release.

Material and Methods

Pancreases were removed from fully fed, male albino rats (approximately 250–300 g body weight) and placed in an open circuit extracorporeal perfusion unit, as described in detail elsewhere [5]. The perfusate consisted of a Hepes buffer (25 mmol/l; pH 7.4) with the following ionic composition: Na^+ 130, K^+ 5, Mg^{2+} 1 and Cl^- 122 mmol/l. The use of a Hepes- as distinct from bicarbonate-buffered medium allowed the use of Ca^{2+} in high concentrations without causing precipitation of Ca^{2+} salts. In view of the results reported by Hellmann [4], no compensation for the changes in osmotic pressure were made, the osmolarity varying between 313 (Ca^{2+} 10 mmol/l) and 337 (Ca^{2+} 18 mmol/l) mosmol/l. The perfusate also contained albumin (5 mg/ml; Fraction V; Sigma Chemicals Co., St. Louis, Mo), dextran (40 mg/ml; T70; Pharmacia Fine Chemicals, Uppsala, Sweden) and as required glucose, theophylline, verapamil (Knoll A.G., Ludwigshafen, Germany), somatostatin (G.I.F.; linear synthetic lyophilized; I. F. Serono, Rome, Italy) and cytochalasin B (Aldrich Chemical Co., Milwaukee, Wisconsin), which was dissolved in dimethylsulphoxide (final concentration of the solvent: 0.1%, *V/V*). The immunoassay method for insulin is detailed elsewhere [5]. The rates of insulin secretion are expressed as $\mu\text{U/min}$ per pancreas.

Fig. 1. Dose-action relationship for Ca^{2+} -induced insulin release in the isolated perfused rat pancreas. Lower panel: insulin release by the isolated perfused rat pancreas exposed for 24 min to a Ca^{2+} -free medium and from the 25th to the 40th min to media containing increasing concentrations of Ca^{2+} (10 mmol/l: closed triangles; 12 mmol/l: closed squares; 13.5 mmol/l: closed circles; and 18 mmol/l: open circles). Upper panel: insulin release is expressed as the integrated output over the 16 min of perfusion following the introduction of extracellular Ca^{2+} . Mean values ($\pm$ SEM) are shown together with the number of individual experiments (n)

Results

Dose-Action Relationship for Ca^{2+} -Induced Insulin Release

After perfusion for 25 minutes with a solution free of Ca^{2+} , the pancreases were exposed to increasing concentrations of extracellular Ca^{2+} (Fig. 1). The

threshold concentration for the stimulatory action of Ca^{2+} lay between 10 and 12 mmol/l. The dose-action relationship for Ca^{2+} -induced insulin release is illustrated in the upper part of Figure 1, which refers to the integrated output of insulin over 16 minutes exposure to various Ca^{2+} concentrations.

Pattern of Ca^{2+} -Induced Insulin Release

At the highest extracellular Ca^{2+} concentrations, the increase in insulin output was detectable within 1–2 minutes of exposure to Ca^{2+} . However, the maximal rate of secretion was not reached until the 10th–16th minutes after the introduction of Ca^{2+} (Fig. 1). Such a maximal secretory rate represents at least twice that evoked by glucose (16.7 mmol/l) at close to physiological Ca^{2+} concentration (2 mmol/l). The progressive build-up in secretory rate during the initial period of exposure to Ca^{2+} was soon followed by a progressive decline in insulin output (Fig. 2). Eventually the rate of secretion returned to its basal value, which was reached after approximately 35–40 minutes of exposure to Ca^{2+} . The fall in secretory rate during the late period does not imply a total lack of responsiveness of the pancreas, since the addition of glucose (16.7 mmol/l) at the 20th minute of exposure to Ca^{2+} was still able to enhance the output of insulin (see inset of Figure 2).

Inhibition of Ca^{2+} -Induced Insulin Release

When Mg^{2+} (10 mmol/l) was present from the beginning of the perfusion, a complete inhibition of Ca^{2+} -evoked insulin release was observed (Fig. 3 and Table 1). The organic Ca^{2+} -antagonist verapamil, which inhibits Ca^{2+} influx in the pancreatic B-cell [6], also caused inhibition of Ca^{2+} -induced insulin secretion at a concentration of 81 $\mu\text{mol/l}$ (Table 1). Exposure of the pancreas to somatostatin (1.25 $\mu\text{g/ml}$) from time 0 to the 35th minute significantly retarded the Ca^{2+} -evoked insulin release (Fig. 4). From the 25th to 35th minute of perfusion the integrated insulin output in the presence of somatostatin averaged $48 \pm 8\%$ ($P < 0.02$) of the mean control value found in its absence.

Potentiation of Ca^{2+} -Induced Insulin Release

When administered together with Ca^{2+} , glucose (16.7 mmol/l) caused a dramatic increase in the output of insulin observed at the lowest active concentration of Ca^{2+} (12 mmol/l). Under these experimental conditions an apparently biphasic insulin response was observed. Glucose, at a concentration of 2.7 mmol/l, failed to affect insulin release at normal

Fig. 2. Pattern of Ca^{2+} -induced insulin release. Mean values ($\pm$ SEM) for insulin release evoked by Ca^{2+} 13.5 mmol/l. Same presentation and experimental conditions as in Fig. 1. Inset: the open circles refer to the same experiments as those shown on the main graph; the closed circles refer to experiments in which glucose 16.7 mmol/l was administered from the 55th min of perfusion onwards. The mean output seen between the 53rd and 55th min is taken as the zero reference value

Table 1. Effect of various agents upon Ca^{2+} -induced insulin release by the isolated perfused rat pancreas^a

Line	Ca^{2+} mmol/l	Mg^{2+} mmol/l	Verapamil $\mu\text{mol/l}$	Glucose mmol/l	Theophylline mmol/l	Cytochalasin B $\mu\text{mol/l}$	Insulin output (% of control)	P
1	10.0	1.0	—	—	—	—	6 ± 2 (5)	
2	10.0	1.0	—	—	—	21	16 ± 3 (5) vs 1	$1 < 0.05$
3	12.0	1.0	—	—	—	—	19 ± 3 (5) vs 1	$1 < 0.005$
4	12.0	1.0	—	2.7	—	—	78 ± 10 (6) vs 3	$3 < 0.001$
5	12.0	1.0	—	16.7	—	—	208 ± 16 (6) vs 4	$4 < 0.001$
6	12.0	1.0	—	—	1.4	—	137 ± 14 (6) vs 3	$3 < 0.001$
7	12.0	1.0	—	—	—	21	58 ± 3 (6) vs 3	$3 < 0.001$
8	13.5	1.0	—	—	—	—	62 ± 6 (10) vs 3	$3 < 0.001$
9	13.5	1.0	81.0	—	—	—	37 ± 4 (4) vs 8	$8 < 0.05$
10	18.0	1.0	—	—	—	—	100 ± 8 (7) vs 8	$8 < 0.002$
11	18.0	1.0	8.1	—	—	—	91 ± 15 (4) vs 10	N.S.
12	18.0	1.0	81.0	—	—	—	57 ± 13 (4) vs 10	< 0.02
13	18.0	10.0	—	—	—	—	4 ± 1 (3) vs 10	< 0.001
14	18.0	1.0	—	—	—	21	99 ± 1 (3) vs 10	N.S.

^a Mean values ($\pm$ SEM) for the integrated amount of insulin released by Ca^{2+} between the 25th and 40th min of perfusion are expressed in per cent of the mean control value shown on line 10 (i.e. 18.0 mmol/l Ca^{2+} and 1.0 mmol/l Mg^{2+}). Such a control value averaged 9.5 ± 0.8 mU/pancreas. Also shown are the number of individual experiments (in parentheses) and the statistical significance of changes in insulin output

extracellular Ca^{2+} concentration [5], but was capable of significantly enhancing the release of insulin evoked by Ca^{2+} 12 mmol/l (Fig. 5 and Table 1). Theophylline (1.4 mmol/l) also markedly enhanced the secretion of insulin evoked by Ca^{2+} 12 mmol/l (Table 1). The mould metabolite cytochalasin B (21 $\mu\text{mol/l}$) potentiated insulin release induced by Ca^{2+} 12 mmol/l (Fig. 6), but failed to affect significantly the higher rate of secretion evoked by Ca^{2+} 18 mmol/l (Table 1). Cytochalasin B apparently lowered the threshold value for the stimulatory action of Ca^{2+} on insulin release. Indeed, in the presence of cytochalasin B, a Ca^{2+} concentration of 10 mmol/l

was sufficient to increase insulin output significantly. The total amount of insulin released over the 16 minute period of exposure to Ca^{2+} 10 mmol/l averaged 560 ± 164 μU ($n = 5$) and $1\,474 \pm 320$ μU ($n = 5$) in the absence and presence of cytochalasin B, respectively.

Discussion

We have recently shown that, in the absence of any other secretagogue, Ca^{2+} is able to provoke a short-lived insulin response in the perfused rat pancreas

Fig. 3. Effect of Mg^{2+} on Ca^{2+} -induced insulin release. The control experiments in which Ca^{2+} 18 mmol/l was introduced at the 25th min of perfusion are shown by the open circles. Same presentation and experimental conditions as in Fig. 1. The data obtained in the presence of Mg^{2+} (10 mmol/l) are given by the closed circles

Fig. 4. Effect of somatostatin on Ca^{2+} -induced insulin release. The control experiments in which Ca^{2+} 18 mmol/l was introduced at the 25th min of perfusion are shown by the open circles. Same presentation and experimental conditions as in Fig. 1. The data obtained when somatostatin (1.25 μ g/ml) was present from time zero until the 35th min are shown by the closed circles

Fig. 5. Effect of glucose upon Ca^{2+} -induced insulin release. The control experiments in which Ca^{2+} 12 mmol/l was introduced at the 25th min of perfusion are shown by the open circles. Same presentation and experimental conditions as in Fig. 1. The data obtained when glucose was introduced together with Ca^{2+} are shown by the closed circles (glucose 2.7 mmol/l: middle curve; glucose 16.7 mmol/l: upper curve)

Fig. 6. Effect of cytochalasin B on Ca^{2+} -induced insulin release. The control experiments in which Ca^{2+} 12 mmol/l was introduced at the 25th min of perfusion are shown by the closed circles. Same presentation and experimental conditions as in Fig. 1. The data obtained in the presence of cytochalasin B (21 μ mol/l) are shown by the open circles

first exposed to the Ca^{2+} -chelating agent (EGTA [3]. Hellman, using a Hepes-buffered medium, was able to provide even more direct evidence for the stimulatory role of Ca^{2+} , showing that under suitable experimental conditions pretreatment with

a Ca^{2+} -deficient medium is not a pre-requisite for the divalent cation to induce insulin release [4]. The data presented in this report confirm that extracellular Ca^{2+} concentrations, above a threshold value of 10 mmol/l, provoke a dose-dependent stimula-

tion of insulin secretion. In contrast with the brief spike of insulin release obtained after pretreatment with EGTA [3], a progressive build-up and monophasic insulin response was observed.

The reason why a sustained secretion of insulin failed to occur is not evident. It could be due to an untoward metabolic effect, since after prolonged exposure (90 min) to a high Ca^{2+} concentration (20 mmol/l), glucose fails to exert its usual stimulant action on $^{45}\text{Ca}^{2+}$ net uptake by isolated islets (unpublished observations). The assumption that an increase in the size of the functionally active pool(s) of Ca^{2+} above a certain optimal value could induce *per se* a diminished insulin secretory response seems untenable [8]. Indeed, glucose, which is known to cause an increase in the total net uptake of Ca^{2+} by the B-cell, remained capable of enhancing insulin output at the very time during perfusion when the hormonal release was falling towards its basal level.

The insulinotropic effect of Ca^{2+} could be due to the fact that high extracellular Ca^{2+} concentrations favour the cytosolic accumulation of this cation, ultimately triggering the secretory response. Unpublished observations have shown that, over 90 minutes incubation in the absence of glucose, the net uptake of $^{45}\text{Ca}^{2+}$ by isolated islets dramatically increases, from 1.34 ± 0.10 to 9.52 ± 0.74 pmol/islet ($n = 13$ in each case), as the Ca^{2+} concentration of the HEPES-buffered incubation medium is raised from 5 to 20 mmol/l.

It is likely that, in the process of Ca^{2+} -induced insulin release, the rate of Ca^{2+} entry in the B-cell plays a critical role. The latter hypothesis is supported by the finding that Ca^{2+} -induced insulin release is inhibited at high extracellular Mg^{2+} concentrations. We have previously shown that Mg^{2+} is a potent inhibitor of Ca^{2+} entry in the B-cell [9], probably by competing with Ca^{2+} for a common cationic carrier-system located within the B-cell plasma membrane. The same competitive effect of Mg^{2+} is observed in the case of Ba^{2+} uptake by the B-cell [10]. The organic Ca^{2+} -antagonist verapamil also inhibits Ca^{2+} -induced insulin release. It is thus plausible that under the present experimental conditions, Ca^{2+} enters the B-cell at the intervention of a native ionophoretic system which, like the exogenous ionophore A23187, would act by virtue of its verapamil-sensitive Ca^{2+} -binding sites [7]. We have indeed recently observed that verapamil inhibits the capacity of the ionophore A23187 to provoke Ca^{2+} translocation in an artificial model [11]. Verapamil could also interfere with the Ca^{2+} -binding sites of the native ionophoretic systems located in the B-cell plasma membrane and, by doing so, inhibit the entry of Ca^{2+} in the B-cell.

It has been proposed by various investigators that the effects of somatostatin on hormonal discharge are related to an alteration in the handling of or response to Ca^{2+} [12–14]. This hypothesis is compatible with the wide-spread inhibitory action of somatostatin on hormone release [15–21] and the role of Ca^{2+} as a cellular messenger in various secretory cells [22]. The data here obtained with somatostatin are also in good agreement with such a hypothesis. Exposure of the pancreas to somatostatin did significantly retard the secretory response to Ca^{2+} . It was recently shown that somatostatin (2 $\mu\text{g/ml}$) significantly decreases glucose-induced $^{45}\text{Ca}^{2+}$ -uptake by isolated islets [14].

The potentiating effect of glucose upon Ca^{2+} -induced insulin release is consistent with the fact that this hexose increases the net uptake of Ca^{2+} by the B-cell. Our finding that a 2.7 mmol/l concentration of glucose was sufficient to enhance Ca^{2+} -induced insulin release would suggest that this sugar is able to affect Ca^{2+} -handling even when used at a concentration far below that normally required to stimulate insulin release. This is not surprising since the rate of glycolysis, which exerts a tight control upon Ca^{2+} net uptake by the B-cell [23], is indeed higher in the presence of glucose 2.7 mmol/l than in the absence of glucose. Moreover, the efflux of $^{45}\text{Ca}^{2+}$ from perfused islets is slightly but significantly reduced by glucose in a concentration (2.5 mmol/l) lower than that required to stimulate insulin release at a physiological concentration of extracellular Ca^{2+} [24].

Theophylline also enhanced Ca^{2+} -evoked insulin release, a finding which is in good agreement with the data obtained by Hellman with isobutylmethylxanthine [4]. These phosphodiesterase inhibitors augment the cyclic-AMP content of the B-cell [8, 25] and, by doing so, may cause a redistribution of Ca^{2+} ions within the B-cell [26]. Since cyclic-AMP is known to inhibit the net uptake of Ca^{2+} by B-cell subcellular particulate fractions [27], our findings suggest that the ability of mitochondria and/or other organelles to store divalent cations plays an important counter-regulatory role in modulating the magnitude of the secretory responses to either Ca^{2+} or Ba^{2+} [10].

Taken as a whole, the present data are compatible with the idea that a rise in the extracellular Ca^{2+} concentration, by increasing Ca^{2+} entry and/or inhibiting Ca^{2+} efflux, provokes a sufficient accumulation of Ca^{2+} in the B-cell to stimulate insulin release. It has been proposed that the cytosolic accumulation of Ca^{2+} triggers insulin secretion by activating a microtubular-microfilamentous effector-system responsible for the exocytosis of secretory

granules. Our findings with cytochalasin B reinforce this concept, since Ca^{2+} -induced insulin release is itself enhanced by this metabolite. As already reported in the case of glucose- or leucine-induced insulin secretion [5, 28], cytochalasin B not only potentiates the secretory response to the secretagogues but also lowers the threshold concentration at which insulinotropic agents become able to evoke insulin release. The mould metabolite may thus sensitize the response of the effector system to sub-threshold concentrations of cytosolic Ca^{2+} [29].

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References

- Malaisse, W.J., Herchuelz, A., Levy, J., Somers, G., Devis, G., Ravazzola, M., Malaisse-Lagae, F., Orci, L.: Insulin release and the movements of calcium in pancreatic islets. In: E. Carafoli, F. Clementi, W. Drabikowski, A. Margreth (Eds.): *Calcium Transport in Contraction and Secretion*, pp. 211–226. Amsterdam: North-Holland Publishing Company 1975
- Malaisse, W.J.: Insulin secretion: multifactorial regulation for a single process of release. *Diabetologia* **9**, 167–173 (1973)
- Devis, G., Somers, G., Malaisse, W.J.: Stimulation of insulin release by calcium. *Biochem. Biophys. Res. Commun.* **67**, 525–529 (1975)
- Hellman, B.: Stimulation of insulin release after raising extracellular calcium. *FEBS Lett.* **63**, 125–128 (1976)
- Van Obberghen, E., Somers, G., Devis, G., Vaughan, G.D., Malaisse-Lagae, F., Orci, L., Malaisse, W.J.: Dynamics of insulin release and microtubular-microfilamentous system. I. Effect of cytochalasin B. *J. Clin. Invest.* **52**, 1041–1051 (1973)
- Levy, J., Herchuelz, A., Sener, A., Malaisse, W.J.: Inhibition by verapamil of calcium influx in the B-cell. *Diabetes* **24**, 400 (1975)
- Somers, G., Devis, G., Malaisse, W.J.: Analogy between native and exogenous ionophores in the pancreatic B-cell. *FEBS Lett.* **66**, 20–22 (1976)
- Hellman, B.: The significance of calcium for glucose stimulation of insulin release. *Endocrinology* **97**, 392–398 (1975)
- Malaisse, W.J., Devis, G., Herchuelz, A., Sener, A., Somers, G.: Calcium antagonists and islet function. VIII. The effect of magnesium. *Diab. Métab. (Paris)* **2**, 1–4 (1976)
- Somers, G., Devis, G., Van Obberghen, E., Malaisse, W.J.: Calcium-antagonists and islet function. VI. Effect of barium. *Pflügers Arch.* **365**, 21–28 (1976)
- Malaisse, W.J., Devis, G., Somers, G.: Inhibition by verapamil of ionophore-mediated calcium translocation. *Experientia* (in press) (1977)
- Curry, D.L., Bennett, L.L.: Reversal of somatostatin inhibition of insulin secretion by calcium. *Biochem. Biophys. Res. Commun.* **60**, 1015–1019 (1974)
- Fujimoto, W.Y., Ensink, J.W.: Somatostatin inhibition of insulin and glucagon secretion in rat islet culture: reversal by ionophore A 23187. *Endocrinology* **98**, 259–262 (1976)
- Bhatena, S.J., Perrino, P.V., Voyles, N.R., Smith, S.S., Wilkins, S.D., Coy, D.H., Schally, A.V., Recant, L.: Reversal of somatostatin inhibition of insulin and glucagon secretion. *Diabetes* **25**, 1031–1040 (1976)
- Brazeau, P., Vale, W., Burgus, R., Ling, N., Burcher, M., River, J., Guillemin, R.: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* **179**, 77–79 (1973)
- Weeke, J., Prange-Hansen, A., Lundbaek, K.: The inhibition by somatostatin of the thyrotropin response to thyrotropin-releasing hormone in normal subjects. *Scand. J. Clin. Lab. Invest.* **33**, 101–103 (1974)
- Tyrrell, J.B., Lorenzi, M., Gerich, J.E., Forsham, P.H.: Inhibition by somatostatin of ACTH secretion in Nelson's syndrome. *J. Clin. Endocrinol. Metab.* **40**, 1125–1127 (1975)
- Bloom, S.R., Mortimer, C.M., Thorner, M.O., Besser, G.M., Hall, R., Gomez-Pau, A., Roy, V.M., Russel, R.C.G., Coy, D.H., Kastin, H.J., Schally, A.V.: Inhibition of gastrin and gastric acid secretion by growth hormone release-inhibiting hormone. *Lancet* **1974 II**, 1106–1109
- Gerich, J.E., Lovinger, R., Grodsky, G.M.: Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline: evidence for a preferential effect on glucagon secretion. *Endocrinology* **96**, 749–754 (1975)
- Johnson, D.G., Ensink, J.W., Koerker, D., Palmer, J., Goodner, C.J.: Inhibition of glucagon and insulin secretion by somatostatin in the rat pancreas perfused in situ. *Endocrinology* **96**, 370–374 (1975)
- Curry, D.L., Bennett, L.L., Li, C.H.: Direct inhibition of insulin secretion by synthetic somatostatin. *Biochem. Biophys. Res. Commun.* **38**, 885–887 (1974)
- Rasmussen, H.: Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science* **170**, 404–412 (1970)
- Sener, A., Levy, J., Malaisse, W.J.: The stimulus-secretion coupling of glucose-induced insulin release. Does glycolysis control calcium transport in the B-cell? *Biochem. J.* **156**, 521–525 (1976)
- Herchuelz, A., Mahy, M.: Influence of sub-threshold glucose concentrations and theophylline on the rapidity of glucose-induced calcium accumulation and exocytotic release in perfused islets. *Diabetologia* **10**, 369 (1974)
- Charles, M.A., Lanecki, J., Pictet, R., Grodsky, G.M.: Insulin secretion. Interrelationships of glucose, cyclic adenosine 3':5'-monophosphate, and calcium. *J. Biol. Chem.* **250**, 6134–6140 (1975)
- Malaisse, W.J.: Effects of cyclic AMP on B-cell function. In: J.E. Dumont, B.L. Brown, N.J. Marshall (Eds.): *Eukaryotic Cell Function and Growth*, pp. 633–638. New York: Plenum Publishing Corporation 1976
- Howell, S.L., Montague, W., Tyhurst, M.: Calcium distribution in islets of Langerhans: a study of calcium concentrations and of calcium accumulation in B-cell organelles. *J. Cell. Sci.* **19**, 395–409 (1975)
- Levy, J., Herchuelz, A., Sener, A., Malaisse-Lagae, F., Malaisse, W.J.: Cytochalasin B-induced impairment of glucose metabolism in islets of Langerhans. *Endocrinology* **98**, 429–437 (1976)
- Malaisse, W.J., Pipeleers, D.G., Van Obberghen, E., Somers, G., Devis, G., Marichal, M., Malaisse-Lagae, F.: The glucoreceptor mechanism in the pancreatic beta-cell. *Am. Zool.* **13**, 605–612 (1973)

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