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Research letter

Insulin secretion in response to high extracellular calcium is not a pathognomonic feature of insulinoma cells

Introduction

Selective arterial calcium stimulation (SACST) with hepatic venous sampling is a powerful test for preoperative localization of insulinomas [1,2]. The test is considered positive when selective calcium injection into one of the major pancreatic arteries causes a more than two-fold increase of plasma insulin in blood samples obtained from hepatic veins. Its clinical specificity is based on the premise that insulinoma β-cells secrete insulin in response to high extracellular calcium whereas normal β-cells do not [1,2]. This assumption however stems from an in vitro study in which human insulinoma cells were compared with MIN6 cells, a mouse cell line secreting insulin [3]. Another study has identified calcium-sensing receptors in human β-cells and suggested that their activation by high extracellular $CaCl_2$ paradoxically inhibits insulin secretion [4]. We previously reported that most human insulinomas indeed secrete insulin when challenged with high $CaCl_2$ in vitro [5]. In the present communication, we show that normal human β-cells within isolated islets share the same property and we discuss why this similarity in behaviour calls for a revision of the mechanistic interpretation but does not invalidate the clinical test.

Methods

The experiments were carried out with islets isolated from 5 nondiabetic multi-organ donors with a mean age of 54.4 years (range 48–65) and a mean BMI of 27.8 kg/m^2 (22–33). The average purity of islet preparations was 79% (75–80). Approval of the experimental use of human islets was granted by the ethics committees of the Medical Faculty of the University of Louvain and the French Minister of Health and Education, and consent was given by the donors' families.

The procedures of islet perifusion to measure the dynamics of insulin secretion have been described [6]. The medium was a bicarbonate-buffered solution continuously gassed with a mixture of O_2:CO_2 and supplemented with 1 mg/mL BSA. The concentration of $CaCl_2$ was 1.25 mmol/L except during 10-min periods of stimulation with 5–10 mmol/L $CaCl_2$ (Ca5 and Ca10). Compound R568, an allosteric agonist of the human calcium-sensing receptor [7], was obtained from Tocris and added from a concentrated stock solution in DMSO. Insulin was measured by a double-antibody radioimmunoassay in effluent fractions collected every 2 min and in acid-ethanol extracts of islets recovered at the end of perifusion [6]. To avoid that calcium precipitates in frozen/thawed solutions interfere with the assay, 3.75 and 8.75 mmol/L EGTA was added to samples containing Ca5 and Ca10, respectively.

Fractional insulin secretion rate was calculated as the percentage of insulin content secreted per minute, which is independent of differences in islet number or insulin content between experiments [6]. Results are presented as means ± SD and the statistical significance of differences was assessed by Anova or *t*-test.

Results

Upon stimulation with 15 mmol/L glucose (G15), all islet preparations used in this study showed biphasic insulin responses (Fig. 1A) similar to those reported in larger series [6]. The increment of insulin secreted during the first phase (10 min) averaged 0.47 ± 0.20% of the islet insulin content. When the same islet preparations were perifused with G4, a 10-min pulse of Ca10 consistently evoked a large and reversible secretion of insulin (Fig. 1B).

The mean insulin response to Ca10 applied in G4 is shown in Fig. 1C. Qualitatively similar responses were observed when pulses of Ca5 or Ca10 were applied in G5 + forskolin (used to increase islet cAMP). The increment of secretion averaged 0.42 ± 0.15, 0.58 ± 0.24 and 0.75 ± 0.20% of islet content during the 3 successive Ca pulses ($P < 0.05$ or less, Anova for paired comparisons). However, the fold-increase was not different (7.5, 6.1 and 6.2×) because of the elevation of the pre-stimulatory secretion rate in G5 + Fk compared with G4. The magnitude of the response to Ca10 in G4 was similar to that of the first phase of the response to G15.

Inhibiting voltage-gated calcium channels with nimodipine prevented the increase in insulin secretion produced by G5 + Fk and reduced, but did not abolish, the responses to high $CaCl_2$ (Fig. 1D). These responses were 60–80% smaller than in controls ($P < 0.01$). Opening ATP-sensitive potassium channels with diazoxide also prevented the effect of G5 + Fk but did not completely block responses to high $CaCl_2$ (Fig. 1E), which were decreased by 70–90% ($P < 0.01$).

Compound R568, an agonist of the extracellular Ca-sensing receptor, produced a small insulin response (0.09 ± 0.02%) in G4 (Fig. 1F). The effect was not augmented in G5 + Fk (0.12 ± 0.05%) but became very large in combination with Ca5 (1.05 ± 0.11%). This combination was about twice as efficient as Ca5 alone (compare Fig. 1F and C).

Discussion

The present study shows that normal human islets respond to pulses of high extracellular $CaCl_2$ by a rapid secretion of insulin rather than by a sustained paradoxical decrease as previously suggested [4]. A similar positive insulin response was previously observed in fragments from normal infant pancreas [8]. Our present finding that stimulation of insulin secretion by $CaCl_2$ was markedly inhibited by nimodipine and diazoxide points to a critical role of Ca^{2+} influx into β-cells through voltage-gated

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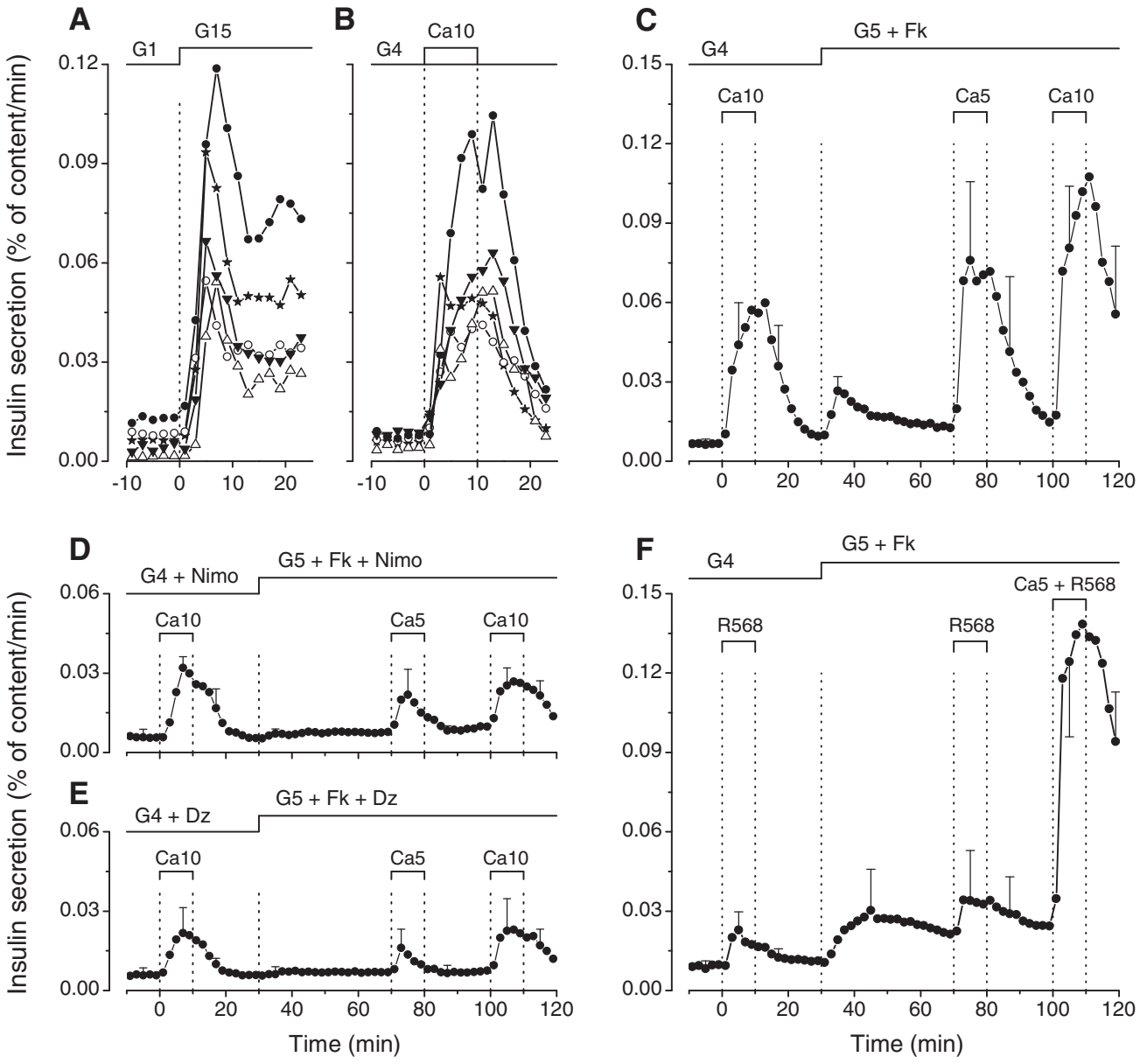

Fig. 1. Insulin secretion induced by glucose and pulses of high extracellular $CaCl_2$ in islets from normal adults. The experiments started with an equilibration period of 60 min, of which only the last 10 min are shown. A. The concentration of glucose was increased from 1 to 15 mmol/L (G1–G15) in a medium containing 2.5 mmol/L $CaCl_2$ throughout. B–F. The medium contained 4 mmol/L glucose (G4) until 30 min and 5 mmol/L glucose plus 1 μmol/L forskolin (G5 + Fk) thereafter. The concentration of $CaCl_2$ was 1.25 mmol/L, except during the pulses with 5 or 10 mmol $CaCl_2$ (Ca5 or Ca10). D. The medium contained 1 μmol/L nimodipine (Nimo) throughout to block voltage-gated calcium channels. E. The medium contained 100 μmol/L diazoxide (Dz) throughout to open ATP-sensitive potassium channels. F. The calcimimetic compound R568 (1 μmol/L) was added as indicated. The third application was combined with Ca5. A–B. Individual insulin responses to glucose and $CaCl_2$ in the five preparations of islets are shown with the same symbols. The other panels show mean values ± SD for 5 (C) and 4 (D–F) islet preparations. Note differences in scale between panels A–B and the others.

calcium channels. However, the incompleteness of these inhibitions suggests involvement of additional mechanisms, among which Ca^{2+} entry via voltage-independent channels is likely. In addition, human β-cells possess functional calcium-sensing receptors [4,7] whose activation, by the calcimimetic R568 and/or by calcium itself, probably contributes to the insulin response. In a previous study, R568 amplified insulin secretion evoked by $CaCl_2$ readmission into a Ca-free medium containing a non-stimulatory concentration of glucose [7].

Since the in vitro insulin response of normal human β-cells to high extracellular $CaCl_2$ is qualitatively similar to that of human insulinoma cells [5], why is SACST negative in pancreatic regions not harbouring an insulinoma in vivo [1,2]? A first possibility is that local calcium concentrations achieved in vivo during the test are lower than those used in vitro. In the absence of direct measurements, intra-pancreatic calcium concentrations can only be estimated from published protocols [1,2]. Pancreatic blood flow is about 2.5 $mL.min^{-1}.g^{-1}$ of tissue [9]. One can thus calculate that blood flow amounts to ~ 75 $mL.min^{-1}$ in the artery supplying the tail region of the organ (~ 30 g). For a subject of 70 kg, a total dose of 0.875 mmol calcium (0.025 mEq/kg × 70) is dissolved in 5 mL (concentration of 175 mmol/L) and injected in less than 10 seconds into the artery [1,2]. After mixing with flowing blood (~ 7 mL of plasma in 10 seconds), a calcium concentration of ~ 75 mmol/L is achieved in blood perfusing the islets, much higher thus than the concentrations used in vitro.

Low blood glucose concentrations, such as those often observed in insulinoma patients, diminish the responsiveness of normal β-cells to various secretagogues. This could contribute to the lack of observable response to SACST in non-insulinoma

regions of the pancreas, but is unlikely to be the sole explanation because this negativity persists when the test is performed during IV glucose infusion to maintain euglycemia [1,10].

In vitro, insulin secretion evoked by a 10-min pulse of high $CaCl_2$ was of similar amplitude to the first phase of the response to G15. Yet, in subjects without insulinoma, only the latter is measurable in sus-hepatic or peripheral blood. The paradox is only apparent and largely linked to differences in the duration of β-cell stimulation in vivo. During SACST, one third only of the pancreas is stimulated by each calcium injection and the stimulation is very short ($<<$ 1 min), whereas the first phase of the response to IV glucose integrates secretion by the whole pancreas over several minutes. Total amounts of secreted insulin are thus very different in vivo.

Conversely, two reasons may explain why the response of an insulinoma is detectable during SACST. The size of insulinomas and their abundance in β-cells are highly variable. In our recent series, the β-cell mass averaged 730 mg (range 155–2080 mg) in insulinomas [5]. The β-cell mass in islets of a normal pancreas is about 900 mg or about 300 mg in the tail. The mass of β-cells is thus often higher in an insulinoma than in islets present in the surrounding third of the pancreas. We also observed that pieces from 6/9 insulinomas strongly secreted insulin when challenged in vitro by a pulse of Ca10 in G5 + Forskolin [5]. The increment above baseline averaged 6.6 ± 1.7% of insulin content, which is 9-fold greater than the response of normal islets (0.75 ± 0.09%). However, the larger fractional secretion is partly counterbalanced by the lower insulin content of insulinoma than normal β-cells [5]. Anyhow, insulinoma cells appear to respond more strongly to $CaCl_2$ than normal β-cells, perhaps because they are more depolarized. Our findings are consistent with reports showing that raising plasma calcium by only 10–20% (through infusion in a peripheral vein) increased plasma insulin levels in insulinoma patients and not in control subjects even when pre-test blood glucose levels were similar [11,12]. Notably, patients with non-insulinoma pancreatogenous hypoglycaemia syndrome, in whom hyperinsulinemic hypoglycaemia is associated with diffuse β-cell hyperplasia, show a positive response to SASCT in all regions of the pancreas [2,13]. This type of diffuse response can probably be explained by a hyperactive state of β-cells.

Conclusion

Normal human β-cells rapidly secrete insulin when challenged by high extracellular $CaCl_2$, a response largely mediated by Ca^{2+} influx through voltage-gated calcium channels. Contrary to widespread assumptions [1,2], the behaviour of normal β-cells is thus qualitatively similar to that of insulinoma cells. Yet, this similarity does not challenge the clinical usefulness of SASCT for localization of insulinomas but entails novel explanations for the selectivity of the test in vivo.

Authors' contribution

JCH designed the study, analysed the data, interpreted the results and wrote the manuscript. FP provided islets and edited the manuscript. MN performed the experiments, analysed the data, and edited the manuscript.

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Disclosure of interest

The authors declare that they have no competing interest.

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J. -C. Henquin[a,*], F. Pattou[b], M. Nenquin[a]
[a]*Unit of Endocrinology and Metabolism, Faculty of Medicine, University of Louvain, 1200 Brussels, Belgium*
[b]*Institut National de la Santé et de la Recherche Médicale U1190, Translational Research for Diabetes, University of Lille, 59000 Lille, France*

*Corresponding author.
E-mail address: jean-claude.henquin@uclouvain.be (J.C. Henquin).

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