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# Effects of Various Modifiers of Insulin Release on the Lanthanum-Nondisplaceable $^{45}Ca^{2+}$ Uptake by Isolated Pancreatic Islets

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**Summary.** The uptake of $^{45}Ca^{2+}$ by a lanthanum-nondisplaceable pool in pancreatic islets was studied. Raising the extracellular D-glucose concentration from 3 to 20 mM stimulated the $^{45}Ca^{2+}$ uptake in hand-dissected islets of ob/ob-mice as well as in collagenase-isolated islets of ob/ob or normal mice. The effect was dose-dependent in the range of 0–20 mM D-glucose and was seen throughout a wide range of extracellular calcium concentrations (16 μmol – 2.56 mmol of $Ca^{2+}$ added per litre of medium). The $^{45}Ca^{2+}$ uptake was also enhanced by other known insulin secretagogues (D-mannose, L-leucine, tolbutamide) and was uninfluenced by compounds lacking insulin-releasing capacity (3-O-methyl-D-glucose, L-glucose, D-galactose, D-leucine). The stimulatory effect of D-glucose was blocked by inhibitors of glucose-induced insulin release (D-mannoheptulose, diazoxide, L-adrenaline). The results support the view that the lanthanum-nondisplaceable calcium pool is related to the insulin-releasing mechanism, although the exact nature of this relationship is still unclear.

**Key words:** Calcium uptake, islets of Langerhans, insulin release, ob/ob-mice.

The stimulatory effect of D-glucose on $^{45}Ca^{2+}$ incorporation into pancreatic islets has been described [9, 11, 15]. Using lanthanum ions to displace the extracellular calcium and to prevent leakage of the intracellular label after incubation with $^{45}Ca^{2+}$, it was shown that the D-glucose-stimulated isotope uptake reflects a net flow of calcium into the islet cells [9]. Cell fractionation experiments suggested that the insulin secretory granules participate in the sequestering of the calcium taken up in response to D-glucose [3]. The present study was undertaken to characterize further the lanthanum-nondisplaceable calcium pool by comparing hand-dissected ob/ob-mouse islets with collagenase-isolated islets of normal mice, by defining the dose-response relationships for D-glucose and extracellular calcium, and by studying the influence of several other modifiers of insulin release.

## Materials and Methods

### *General*

Adult non-inbred ob/ob-mice or lean mice of normal phenotype were taken from the Umeå colony and starved overnight. Pancreatic islets were usually microdissected free-hand from ob/ob-mice with the pancreas immersed in basal medium at 2° C. The basal medium in microdissection and subsequent incubations was a salt-balanced tris buffer, the detailed composition of which has been described [9]. In some experiments, islets from ob/ob-mice and lean mice were obtained by collagenase digestion of the pancreas in Hank's solution; these islets, too, were then incubated in tris buffer. All incubations were performed at 37° C in media equilibrated with ambient air.

### *$^{45}Ca^{2+}$ Uptake*

Batches of five islets from ob/ob-mice, or ten from the normal mice, were incubated for 2 h in 200 μl of basal medium labelled with $^{45}Ca^{2+}$ (7.8 Ci/mol of $Ca^{2+}$).

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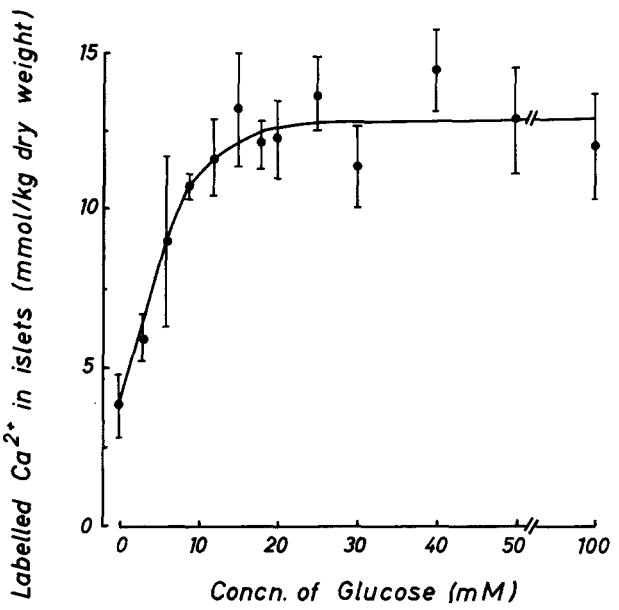


**Fig. 1.** Effects of various concentrations of D-glucose on the lanthanum-nondisplaceable $^{45}Ca^{2+}$ uptake. After preliminary incubation for 30 min in basal medium containing 3 mM D-glucose, microdissected ob/ob-mouse islets were incubated for 120 min in medium labelled with $^{45}Ca^{2+}$ and supplemented with D-glucose as indicated. The islet radioactivity after washing with lanthanum is presented in terms of mmol of calcium with the same specific activity as in the incubation medium. Mean values ± SE of 4–5 different experiments

**Table 1.** D-glucose-stimulated $^{45}Ca^{2+}$ uptake in different types of isolated islets

| Islet model | No. of expts. | Uptake of labelled calcium (mmol/kg dry islet) | | |
|---|---|---|---|---|
| | | 3 mM glucose (a) | 20 mM glucose (b) | Difference (b)–(a) |
| Microdissected ob/ob-mouse islets | 11 | 4.04±0.79 | 10.40±0.49 | 6.20±0.94[b] |
| Collagenase isolated ob/ob-mouse islets | 7 | 2.40±0.44 | 9.78±0.98 | 7.39±1.02[b] |
| Collagenase-isolated normal mouse islets | 8 | 4.49±0.73 | 9.48±0.90 | 5.00±1.32[a] |

Islets from ob/ob or normal mice were isolated by free-hand microdissection or collagenase digestion. After preliminary incubation for 30 min in basal medium containing 3 mM D-glucose, the islets were incubated for 120 min in medium labelled with $^{45}Ca^{2+}$ and containing 3 or 20 mM D-glucose. The radioactivity retained after washing with lanthanum is expressed in terms of mmol of calcium with the same specific activity as in the incubation medium. Mean values ± SE. The effect of D-glucose was evaluated by t-testing the differences between parallel incubations

[a] $p < 0.05$

[b] $p < 0.001$

This incubation was followed by 1 h of washing with non-radioactive basal medium, supplemented with 2 mM $LaCl_3$. The rationale of this technique [4] and its application to pancreatic islets [9] have been described. After freeze-drying (−40° C, 0.1 Pa) overnight and weighing on a quartz-fibre balance, the islets were dissolved in hyamine and their radioactivity determined by liquid-scintillation counting. Samples of incubation medium were used as external standards in each experiment.

### *Chemicals*

The sources of chemicals were as follows: — British Drug Houses, Poole, England: D-glucose, $LaCl_3$; Sigma Chemical Co., St. Louis, Mo., U.S.A.: L-adrenaline, D-galactose, L-glucose, D-mannose, 3-O-methyl-D-glucose, D-mannoheptulose, D-leucine, L-leucine; Schering Corp., Bloomfield, N.J., U.S.A.: diazoxide; Farbwerke Hoechst, Frankfurt/M., Germany: tolbutamide; Worthington Biochemical Corporation, Freehold, N.J., U.S.A.: crude collagenase; the Radiochemical Centre, Amersham, England: $^{45}CaCl_2$.

## Results

Confirming previous observations [9], an increase of the D-glucose concentration from 3 to 20 mM stimulated the $^{45}Ca^{2+}$ uptake by the lanthanum-nondisplaceable pool in hand-dissected ob/ob-mouse islets (Table 1).

The same effect was also obtained with collagenase-isolated islets, whether from ob/ob or normal mice (Table 1).

The glucose-induced $^{45}Ca^{2+}$ uptake was clearly dose-dependent in the range of 0–20 mM D-glucose, higher glucose concentrations did not increase the $^{45}Ca^{2+}$ uptake further (Fig. 1). The stimulatory effect of 20 mM D-glucose was seen over a range of added extracellular $Ca^{2+}$ concentrations as wide as 16 μM–2.56 mM (Table 2).

The $^{45}Ca^{2+}$ uptake was also increased by 17 mM D-mannose, 17 mM L-leucine, or 1 mM tolbutamide (Table 3). No effect was obtained with 3-O-methyl-D-glucose, D-galactose, L-glucose, or D-leucine, each of which was tested at a concentration of 17 mM (Table 3). The D-glucose-induced $^{45}Ca^{2+}$ uptake was blocked by 10 mM D-mannoheptulose, 125 μg/ml of diazoxide or 0.2 μg/ml of L-adrenaline (Table 4).

**Table 2.** Effect of extracellular calcium on the lanthanum-nondisplaceable $^{45}Ca^{2+}$ uptake

| | Uptake of labelled calcium (mmol/kg dry islet) | | |
|---|---|---|---|
| $Ca^{2+}$ concn. (mM) | Control (3 mM glucose) (a) | Test (20 mM glucose) (b) | (b)–(a) |
| 2.56 | 6.79±0.83 | 13.18±1.06 | 6.39±1.20[b] |
| 1.00 | 3.30±0.45 | 6.57±1.66 | 3.28±1.33 |
| 0.50 | 1.81±0.27 | 4.10±0.41 | 2.29±0.27[c] |
| 0.10 | 0.67±0.07 | 1.77±0.45 | 1.09±0.39[a] |
| 0.05 | 0.53±0.12 | 1.33±0.22 | 0.81±0.17[b] |
| 0.016 | 0.15±0.01 | 0.49±0.11 | 0.34±0.10[a] |

After preliminary incubation for 30 min in medium containing 3 mM D-glucose and non-radioactive calcium as indicated, microdissected ob/ob-mouse islets were incubated for 120 min with the same concentration of calcium in medium labelled with $^{45}Ca^{2+}$ and containing 3 or 20 mM D-glucose. The calcium concentrations refer to the amounts of salt added to the medium. The results of 4 different experiments are presented as in Table 1

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

**Table 3.** Lanthanum-nondisplaceable $^{45}Ca^{2+}$ uptake by islets exposed to various insulin secretagogues and compounds lacking effect on insulin release

| | | Uptake of labelled calcium (mmol/kg dry islet) | | |
|---|---|---|---|---|
| Test substance | No. of expts. | Control (a) | Test (b) | (b)–(a) |
| D-glucose | 11 | 4.04±0.79 | 10.40±0.49 | 6.20±0.94[c] |
| D-mannose | 11 | 4.04±0.79 | 8.32±0.90 | 4.12±1.17[a] |
| 3-O-methyl-D-glucose | 5 | 4.92±0.72 | 4.76±0.79 | –0.16±1.18 |
| D-galactose | 6 | 5.81±0.36 | 5.09±0.36 | –0.72±0.98 |
| L-glucose | 4 | 5.08±0.35 | 4.37±0.35 | –0.71±1.30 |
| D-leucine | 5 | 3.81±0.44 | 5.21±0.72 | 1.41±0.66 |
| L-leucine | 5 | 3.81±0.44 | 9.34±0.92 | 5.53±0.75[b] |
| Tolbutamide | 6 | 4.35±0.26 | 13.56±0.50 | 9.21±0.72[c] |

After preliminary incubation for 30 min in basal medium containing 3 mM D-glucose, microdissected ob/ob-mouse islets were incubated for 120 min in medium labelled with $^{45}Ca^{2+}$. Control media contained 3 mM D-glucose alone. Test media contained 20 mM D-glucose or 3 mM D-glucose in combination with 17 mM of another sugar or amino acid or 1 mM tolbutamide. Results are presented as in Table 1

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

**Table 4.** Lanthanum-nondisplaceable $^{45}Ca^{2+}$ uptake by islets exposed to inhibitors of insulin release

| | | | Uptake of labelled calcium (mmol/kg dry islet) | | |
|---|---|---|---|---|---|
| Test substance | Glucose concn. (mM) | No. of expts. | Control (a) | Test (b) | (b)–(a) |
| Mannoheptulose (10 mM) | 3 | 6 | 4.57±0.68 | 6.23±1.03 | 1.66±0.76 |
| Mannoheptulose (10 mM) | 20 | 6 | 11.33±1.39 | 5.39±0.90 | –5.94±1.52[a] |
| Diazoxide (125 μg/ml) | 3 | 6 | 3.82±0.31 | 4.00±0.67 | 0.18±0.66 |
| Diazoxide (125 μg/ml) | 20 | 6 | 10.17±1.43 | 4.53±0.49 | –5.64±1.03[b] |
| Adrenaline (0.2 μg/ml) | 3 | 5 | 5.04±0.43 | 5.95±0.77 | 0.91±0.63 |
| Adrenaline (0.2 μg/ml) | 20 | 5 | 11.92±0.58 | 4.58±0.73 | –7.34±0.79[c] |

After preliminary incubation for 30 min in basal medium containing 3 mM D-glucose, microdissected ob/ob-mouse islets were incubated for 120 min in medium labelled with $^{45}Ca^{2+}$. Control media contained 3 or 20 mM D-glucose alone, whereas test media also contained an insulin release inhibitor as indicated. Results are presented as in Table 1

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

## Discussion

Because ob/ob-mouse islets contain at least 90% B-cells, it has been assumed that these cells are responsible for the stimulatory effect of D-glucose on the islet calcium uptake [9]. The experiments reported here extend the observations to collagenase-isolated islets of normal mice, an experimental model more widely used than the hand-dissected ob/ob-mouse islets. The results obtained with normal and ob/ob-mouse islets are very similar, supporting the notion that the behaviour of the B-cells is virtually the same in the two types of mice [8, 12].

A very strict correlation between islet radioactivity and insulin release has been demonstrated in rat islets that were labelled with $^{45}Ca^{2+}$ and then washed with ordinary buffer [14, 15]. Although that technique permits a fraction of the intracellular $^{45}Ca^{2+}$ to escape before the islet radioactivity is counted, the residual $^{45}Ca^{2+}$ probably represents a portion of the lanthanum-nondisplaceable calcium pool measured here [9]. Our results agree with those of Malaisse and coworkers [14, 15] in that the lanthanum-nondisplaceable $^{45}Ca^{2+}$ incorporation was enhanced by insulin secretagogues (D-glucose, D-mannose, L-leucine, tolbutamide), uninfluenced by compounds lacking insulin-releasing activity (3-O-methyl-D-glucose, L-glucose, D-leucine, D-galactose) and counteracted by inhibitors of insulin release (D-mannoheptulose, diazoxide, L-adrenaline). Moreover, the uptake of $^{45}Ca^{2+}$ showed a dependence on D-glucose concentration that was very similar to that of glucose-induced insulin release [7]. These results support the view that the lanthanum-nondisplaceable calcium pool is related to the insulin releasing mechanism.

In spite of the above correlations and the probable location of lanthanum-nondisplaceable calcium in the insulin secretory granules [3] there need not be a strict cause-effect relationship between the lanthanum-nondisplaceable calcium uptake and insulin release. The enhanced calcium uptake may well be an epiphenomenon without direct importance for stimulus-secretion coupling, or it may be just one of several factors participating in this process. Indeed, previous data on the kinetics of the lanthanum-nondisplaceable pool indicate that this pool is too slowly mobilized to explain the fast drop in the secretory rate that occurs upon a decrease of the extracellular calcium or glucose [9]. The acute control of insulin secretion is likely to be more dependent on a mobile calcium pool that is displaceable with lanthanum and, thus, perhaps located in the B-cell plasma membranes [10]. The present results underline the difficulties attending the physiological interpretation of the changes of the lanthanum-nondisplaceable pool. The correlation between $^{45}Ca^{2+}$ uptake and insulin release was not as strict as previously reported for rat islets [14, 15]. Thus, tolbutamide, mannose, and L-leucine are clearly less potent than D-glucose in stimulating the release of insulin from normal mouse islets [1, 2] as well as from the islets of ob/ob-mice [13]. However, these insulin secretagogues appeared equally effective in stimulating the lanthanum-nondisplaceable $^{45}Ca^{2+}$ uptake.

Like Malaisse-Lagae and Malaisse [15] we found that D-glucose stimulated the $^{45}Ca^{2+}$ uptake at extracellular calcium concentrations as low as in the micromolar range. The relative effect of raising the D-glucose concentration from 3 to 20 mM was about the same at all calcium concentrations tested. The absolute amount of calcium taken up, however, fell with the extracellular concentration. These observations indicate that the basal insulin release from isolated islets (below 3 mM D-glucose) is largely independent of the amount of calcium taken up by the lanthanum-nondisplaceable pool. Unlike the secretory response to a high glucose concentration, the basal secretory rate is not drastically decreased when the extracellular calcium concentration is reduced from the physiological level [6].

Although a glucose-induced insulin secretory response is not usually observed in the presence of only micromolar calcium [5, 6], there may be a response of such small magnitude as to escape detection by the methods regularly employed. At an extracellular calcium concentration of 16 μM, the glucose-induced calcium uptake was only about 5% of that seen at the normal calcium concentration (2.56 mM). To rule out the possibility that this small calcium uptake is not due to a corresponding enhancement of insulin release, the measurements of secretion must be sufficiently sensitive as to detect a 5% increase above the basal rate. The random error associated with measurements of insulin release makes it hard to rule out the occurrence of such a small change. In contrast to a previous conclusion [15] we therefore feel that the question remains open as to whether the increased $^{45}Ca^{2+}$ uptake is due to or mediates the effect of glucose on insulin release.

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## References

1. Ashcroft, S. J. H., Bassett, J. M., Randle, P. J.: Insulin secretion mechanisms and glucose metabolism in isolated islets. Diabetes **21**, 538–545 (1972)

2. Ashcroft, S. J. H., Weerasinghe, L. C. C., Randle, P. J.: Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. Biochem. J. **132**, 223–231 (1973)
3. Bloom, G. D., Hellman, B., Sehlin, J., Täljedal, I.-B.: Glucose-stimulated and $La^{3+}$-nondisplaceable $Ca^{2+}$ pool in pancreatic islets. Amer. J. Physiol. (in press)
4. van Breemen, C., Farinas, B. R., Gerba, P., McNaughton, E. D.: Excitation-contraction coupling in rabbit aorta studied by the lanthanum method for measuring cellular calcium influx. Circulat. Res. **30**, 44–54 (1972)
5. Curry, D. L., Bennett, L. L., Grodsky, G. M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology **83**, 572–584 (1968)
6. Hellman, B.: The significance of calcium for glucose stimulation of insulin release. Endocrinology **97**, 392–398 (1975)
7. Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., Täljedal, I.-B.: Iodoacetamide-induced sensitization of the pancreatic β-cells to glucose stimulation. Biochem. J. **132**, 775–789 (1973)
8. Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., Täljedal, I.-B.: The pancreatic β-cell recognition of insulin secretagogues. Effects of calcium and sodium on glucose metabolism and insulin release. Biochem. J. **138**, 33–45 (1974)
9. Hellman, B., Sehlin, J., Täljedal, I.-B.: Effects of glucose on $^{45}Ca^{2+}$ uptake by pancreatic islets as studied with the lanthanum method. J. Physiol. (Lond.) **254**, 639–656 (1976)
10. Hellman, B., Sehlin, J., Täljedal, I.-B.: Calcium and secretion: distinction between two pools of glucose-sensitive calcium in pancreatic islets. Science (in press)
11. Henquin, J.-C., Lambert, A. E.: Cobalt inhibition of insulin secretion and calcium uptake by isolated rat islets. Amer. J. Physiol. **228**, 1669–1677 (1975)
12. Idahl, L.-Å.: A micro perifusion device for pancreatic islets allowing concomitant recordings of intermediate metabolites and insulin release. Analyt. Biochem. **50**, 386–398 (1972)
13. Lernmark, Å: Specificity of leucine stimulation of insulin release. Hormones **3**, 14–21 (1972)
14. Malaisse, W. J.: Role of cations. In: Handbook of experimental pharmacology, vol. XXXII/2. Insulin II (eds. A. Hasselblatt, F. v. Bruchhausen), pp. 145–155. Berlin, Heidelberg: Springer-Verlag 1975
15. Malaisse-Lagae, F., Malaisse, W. J.: Stimulus-secretion coupling of glucose-induced insulin release. III. Uptake of $^{45}$calcium by isolated islets of Langerhans. Endocrinology **88**, 72–80 (1971)

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