ANALOGY BETWEEN NATIVE AND EXOGENOUS IONOPHORES IN THE PANCREATIC B-CELL

Guido SOMERS, Ghislain DEVIS and Willy J. MALAISSE
Laboratory of Experimental Medicine, Brussels Free University, B-1000 Brussels, Belgium

Received 29 April 1976

1. Introduction

A sufficient influx of Ca^{2+} into the pancreatic B-cell is known to usually play a permissive role in the maintenance of the insulin secretory process [1]. This inward movement of Ca^{2+} across the B-cell membrane is thought to be mediated by a finite number of carriers, the relationship between the rate of Ca^{2+} entry and extracellular Ca^{2+} concentration being described by the Michaelis-Menten equation [2]. In the B-cell, this native ionophore system is further characterized by competition between Ca^{2+}, Mg^{2+}, Co^{2+} and Ba^{2+} [3–5] and by its sensitivity to organic Ca^{2+}-antagonists, such as verapamil and D600 [6,7]. The data presented in this report suggest that the native Ca^{2+}-carrier system of the pancreatic B-cell also behaves in a mannner analogous to A23187, an antibiotic ionophore from streptomyces [8] that is known to promote insulin release [9–13].

2. Materials and methods

Pancreases removed from fully fed albino rats were placed in an open circuit extracorporeal perfusion unit, as described in detail elsewhere [14]. The perfusate was a bicarbonate-buffered solution containing albumin (5 mg/ml; Fraction V; Sigma Chemicals Co, St. Louis, MO), dextran (40 mg/ml; T 70; Pharmacia Fine Chemicals, Uppsala, Sweden), glucose (0.5 mg/ml) and, as required EGTA (ethyleneglycol-bis [β-amino-ethyl ether] *N,N'*-tetra-acetic acid neutralized to pH 7 with NaOH), verapamil (Knoll A. G.) and the ionophore A23187 (kindly donated by Dr M. Root; Eli Lilly Company, Indianapolis, Ind). A23187 was first dissolved in ethanol (initial concentration 0.5 mg/ml). The ionic composition of the perfusate was as follows: Na^+ 139, K^+ 5, Cl^- 120 and HCO_3^- 24 meq/l. The total calcium concentration, measured by atomic absorption spectroscopy [15], averaged 0.25 ± 0.02 mM (n = 8). Higher Ca^{2+} concentrations could not be used because, in the presence of A23187, they provoked an immediate and important pancreatic oedema with a marked decrease in perfusion flux. No Mg^{2+} was added to the perfusate in order to facilitate interpretation of the experimental data obtained with A23187, which is known to display almost identical affinities towards Ca^{2+} and Mg^{2+} [8]. The immunoassay method for insulin is detailed elsewhere [16]. The rate of insulin secretion is expressed as μU/min per pancreas.

3. Results and discussion

The administration of ionophore A23187 (10 μM) to the isolated perfused rat pancreas provoked a dramatic but short-lived release of insulin, no change in secretion rate being observed in the control experiments in which only ethanol (10 μl/ml), the solvent for A23187, was added to the perfusate (fig.1, left panel).

The secretory response to A23187 was apparently dependent on the presence of a sufficient amount of extracellular Ca^{2+}, since it was abolished in the presence of EGTA (fig.1, middle panel). This finding is consistent with the view that the ionophore A23187 provokes insulin release by facilitating the inward flux of Ca^{2+} across the B-cell membrane [9–13]

In the absence of EGTA, verapamil provoked a

 North-Holland Publishing Company – Amsterdam

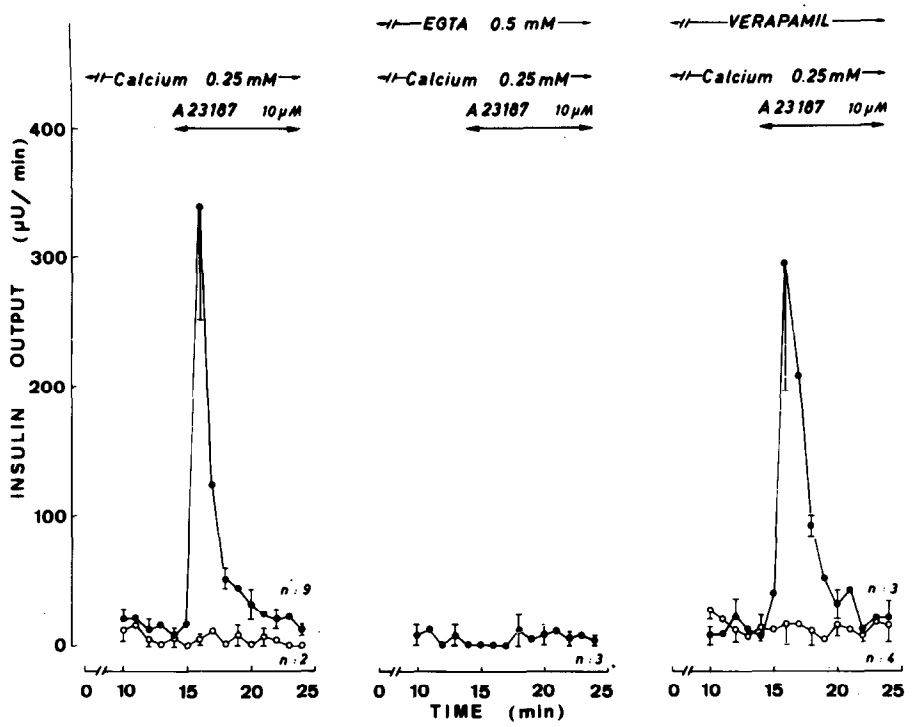

Fig.1. Effect of A23187 upon insulin release by the isolated perfused rat pancreas. (Left panel) The results obtained in the presence of ionophore (closed circles) are compared to those seen when only ethanol (10 μl/ml; open circles) was administered from the 15th to 24th min. (Middle panel) Effect of EGTA (0.5 mM) upon the response to the ionophore. (Right panel) Effect of increasing concentration of verapamil (8.1 μM, closed circles; 81.0 μM, open circles) upon the response to the ionophore. Mean values (± SEM) are given together with the number of individual experiments in each group (n).

dose-dependent inhibition of A23187-induced insulin release (fig.1, right panel). At a low dose (8.1 μM), the inhibitory effect of the Ca^{2+}-antagonist failed to achieve statistical significance ($P > 0.7$), the 16th min secretory peak averaging 340 ± 88 and 296 ± 100 μU/min in the absence and presence of verapamil, respectively. At a higher concentration (81 μM), verapamil completely suppressed the insulinotropic action of A23187. These results are reminiscent of those recently reported by Thorn et al. [17] who observed that D600 (20 μM) reduces the release of vasopressin evoked by A23187 in rat neurohypophyseal slices.

In the pancreatic B-cell, verapamil is known to abolish the release of insulin evoked by glucose, sulfonylurea, Ca^{2+} and Ba^{2+} [6,18,19]. The influence of verapamil upon glucose metabolism, proinsulin biosynthesis and ^{45}Ca handling in isolated islets is compatible with the view that the major effect of the drug is to inhibit Ca^{2+} entry into the B-cell [20]. Within the framework of such a concept, the present results suggest that verapamil may interact with a Ca^{2+}-binding site common to both A23187 and the native ionophore located at the B-cell membrane.

Acknowledgements

We are grateful to Mr G. Schoonjans and Miss G. De Pauw for technical assistance and to Mrs B. Noël for secretarial help. This work was supported in part by grants from the Belgian Foundation for Scientific Medical Research.

References

[1] Malaisse, W. J., Devis, G., Herchuelz, A., Sener, A. and Somers, G. (1976) Diabète et Métabolisme 2, 1.

[2] Herchuelz, A., Levy, J., Mahy, M., Schoonheydt, J. and Sener, A. (1975) Diabetologia 11, 350.
[3] Milner, R. D. G. and Hales, G. N. (1967) Diabetologia 3, 47.
[4] Malaisse, W. J., Brisson, G. and Malaisse-Lagae, F. (1970) J. Lab. Clin. Med. 6, 895.
[5] Henquin, J. and Lambert, A. E. (1975) Amer. J. Physiol. 228, 1669.
[6] Devis, G., Somers, G., Van Obberghen, E. and Malaisse, W. J. (1975) Diabetes 24, 547.
[7] Malaisse, W. J., Devis, G., Pipeleers, D. G. and Somers, G. (1976) Diabetologia 12, 77.
[8] Reed, P. W. and Lardy, H. A. (1972) The Role of Membranes in Metabolic Regulation, pp. 111–131, Academic Press, New York.
[9] Wollheim, C. B., Blondel, B., Trueheart, P. A., Renold, A. E. and Sharp, G. W. G. (1975) J. Biol. Chem. 250, 1354.
[10] Hellman, B. (1975) Biochim. Biophys. Acta 399, 157.
[11] Karl, R. C., Zawalich, W. S., Ferendelli, J. A. and Matschinsky, F. M. (1975) J. Biol. Chem. 250, 4575.
[12] Charles, M. A., Lawecki, J., Pictet, R. and Grodsky, G. M. (1975) J. Biol. Chem. 250, 6134.
[13] Ashley, J. P. and Speake, R. N. (1975) Biochem. J. 150, 89.
[14] Van Obberghen, E., Somers, G., Devis, G., Vaughan, G. D., Malaisse-Lagae, F., Orci, L. and Malaisse, W. J. (1973) J. Clin. Invest. 52, 1041.
[15] Willis, J. B. (1960) Nature 186, 249.
[16] Wright, P. H., Malaisse, W. J. and Reynolds, I. J. (1967) Endocrinology 81, 226.
[17] Thorn, N. A., Russel, J. T. and Robinson, I. C. A. F. (1975) Calcium Transport in Contraction and Secretion, pp. 261–269, North-Holland Publishing Company, Amsterdam.
[18] Devis, G., Somers, G. and Malaisse, W. J. (1975) Biochem. Biophys. Res. Commun. 67, 525.
[19] Devis, G., Somers, G., Van Obberghen, E. and Malaisse, W. J. (1975) Diabetologia 11, 338.
[20] Levy, J., Herchuelz, A., Sener, A. and Malaisse, W. J. (1975) Diabetes 24, 400.