INHIBITION OF GLUCAGON SECRETION BY GLUCOSE AND GLYCERALDEHYDE ON ISOLATED ISLETS OF WISTAR RATS

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1. Introduction

In vivo, glucose stimulated insulin secretion and inhibited glucagon secretion [1]. Whereas the effect of glucose on insulin release is well documented in vitro too, it has been difficult to demonstrate the action of glucose on glucagon release and quite different results were reported [2–10].

Observing that the lack of insulin caused an increased glucagon secretion irrespective of the magnitude of hyperglycemia [5,11–12] and that insulin application restored the A-cell response to glucose [5,11–13] it has been hypothesized that the glucose-mediated inhibition of glucagon secretion is caused by insulin [12].

On the other hand Edwards and Taylor [2] suggested that glucagon secretion can be reduced by energy-yielding fuels and that this suppression is prevented by blocking ATP-formation. To test this hypothesis we investigated the effect of the glucose metabolite D-glyceraldehyde, which mimicked the glucose effect on B-cells in vitro [14–16], on glucagon release and compared with the inhibitory capacity of glucose. It was found that both glucose and glyceraldehyde decreased glucagon release and increased insulin secretion on isolated islets of Wistar rats.

2. Materials and methods

The methods for isolation of islets of Langerhans and for the radioimmunoassay of glucagon and insulin, were similar to those described previously [17–19]. Adult male fed Wistar rats (body weight 250 g) were used in these experiments. The islets were preincubated in Krebs-Ringer-bicarbonate buffer with 16 mM HEPES (*N*-2-hydroxy-ethylpiperazine *N'*-2-ethanesulphonic acid), supplemented with 5 mM glucose, 25 mM sucrose and 1 mg/ml bovine serum albumin for 30 min at 37°C. After this the islets were divided into groups of five, which were incubated in 0.5 ml of the same type of buffer, supplemented with 1 mg/ml bovine serum albumin, and 0–30 mM D-glucose or 0–30 mM D-glyceraldehyde as described in the legends of figures. To avoid any osmotic influences all incubation media were corrected with sucrose up to 30 mM saccharides. After 60 min of incubation samples of buffer were removed and stored (–20°C) until the hormone contents were determined. Because similar sized islets were used throughout these experiments the hormone secretion rates were calculated in ng per islet in 60 min. Statistical differences were checked by Students t-test.

3. Results and discussion

As the results demonstrated glucose (fig. 1) and glyceraldehyde (fig. 2) stimulated insulin secretion in rat islets. Whereas glucose caused the well documented sigmoidal glucose response, this typical shape of secretion pattern could not be observed with glyceraldehyde as a stimulus. Firstly it seems that the glyceraldehyde threshold is lower than that of glucose, because 2.5 mM glyceraldehyde stimulated significantly ($p < 0.01$), and secondly the maximal effect on insulin secretion is reached with 10 mM. Higher glyceraldehyde concentrations resulted in rather a

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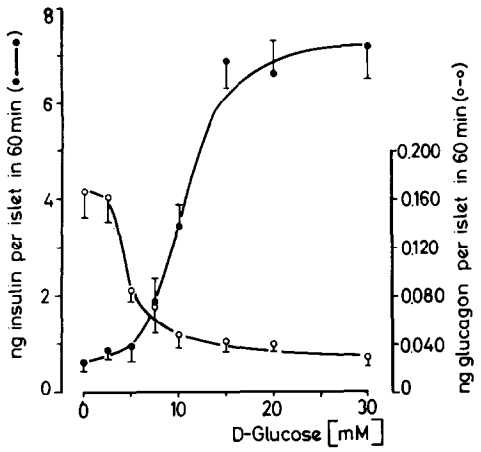

Fig. 1. Insulin and glucagon release of isolated rat islets dependent on D-glucose. After a prestimulatory incubation for 30 min 5 islets pro vial were incubated for 60 min at 37°C with the indicated glucose concentrations corrected with sucrose up to 30 mM saccharides. The secretion rates (ng/islet in 60 min) are expressed as the mean ± SEM of 10 experiments.

tendency to decrease the hormone output than a further increase [14,15], as it was found for glucose. Furthermore the glucose effect on insulin secretion is much more pronounced than that of glyceraldehyde (fig. 3). Both, glucose and glyceraldehyde decreased the glucagon secretion in vitro (fig. 1 and 2). It was remarkable that 5 mM glyceraldehyde (although this

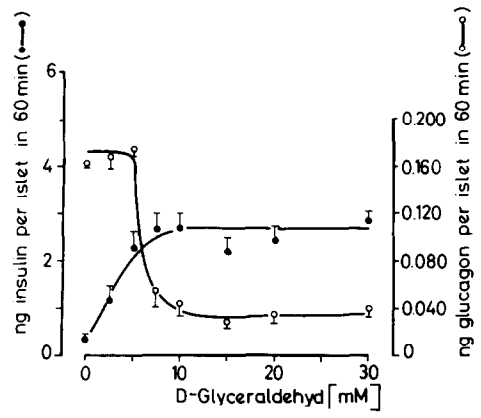

Fig. 2. Influence of D-glyceraldehyde on the release of insulin and glucagon in vitro (for details see legend to fig. 1, number of experiments–8).

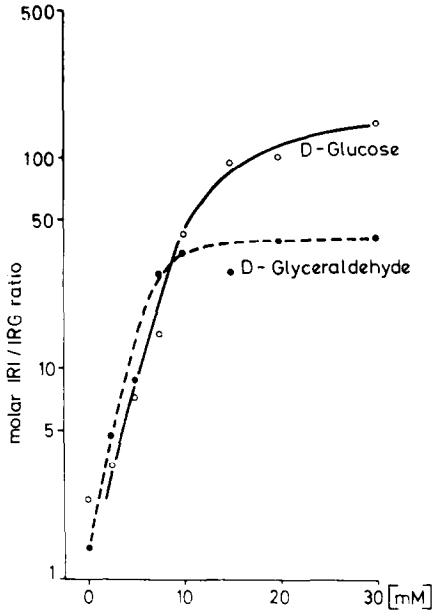

Fig. 3. Molar insulin/glucagon ratio dependent on the concentrations of D-glucose or D-glyceraldehyde. The values were calculated from the mean of experiments depicted in figs. 1 and 2. The following mol. wts for calculation were used: insulin–5777, glucagon–3485.

concentration resulted in an enhanced insulin secretion) did not cause a significant suppression of glucagon release, which already could be observed with 5 mM glucose ($p < 0.02$). 7.5 mM glyceraldehyde (equivalent to 3.75 mM glucose) conditionated a statistical significant inhibitory effect ($p < 0.001$). Higher concentrations of both metabolites (> 10 mM) caused a further slight suppression of glucagon release. The results indicated that the glucagon secretion is at least in part independent of the secreted amount of insulin, because i. 5 mM glyceraldehyde does not altered the glucagon secretion, whereas the insulin secretion is stimulated ($p < 0.001$), ii. 5 mM glucose inhibited glucagon release by an unstimulated insulin release, and iii. the glucagon suppression with both substances was nearly identical, although glucose stimulated insulin secretion more pronouncedly than glyceraldehyde (fig. 3).

Our results demonstrated clearly that glucose and glyceraldehyde influenced the pancreatic hormone secretion in a qualitatively similar manner, and that

this behavior can not be due to osmotic changes in the medium. The results can be explained by the hypothesis that glucose metabolism is essential for recognition of glucose as stimulus of insulin secretion and as inhibitor of glucagon secretion (if there is no absolute lack of insulin) and that the signal compounds are produced at or below the triose phosphate step in the A- and B-cell glycolysis. Whereas this signal compound is amplified in the B-cells resulting in the sigmoidal curve of insulin secretion, it seems that the glucagon secretion is fully inhibited after reaching the threshold level.

References

[1] Unger, R. H. (1972) Handbook of Physiology, Endocrinology I, Endocrine pancreas 529.
[2] Edwards, J. C. and Taylor, K. W. (1970) Biochim. Biophys. Acta 215, 310.
[3] Vance, J. E., Buchanan, K. D., Challoner, D. R. and Williams, R. H. (1968) Diabetes 17, 187.
[4] Buchanan, K. D. and Mawhinney, W. A. A. (1973) Diabetes 22, 797.
[5] Buchanan, K. D. and Mawhinney, W. A. A. (1973) Diabetes 22, 801.
[6] Foá, P. P. (1973) Amer. Zool. 13, 613.
[7] Chesney, T. McC. and Schofield, J. G. (1969) Diabetes 18, 627.
[8] Leclercq, V., Brisson, G. R. and Malaisse, W. J. (1971) Nature, New Biology, 231, 248.
[9] Hahn, H. J., Ziegler, M., Speck, G. A. and Ziegler, B., VIII. Karlsburger Symposium über Frühdiabetes, Berlin 1974, in press.
[10] Marliss, E. B., Wollheim, C. B., Blondel. B., Orci, L., Lambert, A. E., Stauffacher, W., Like, A. A. and Renold, A. E. (1973) Eur. J. Clin. Invest. 3, 16.
[11] Müller, W. A., Faloona, G. R. and Unger, R. H. (1971) J. Clin. Invest. 50, 1992.
[12] Samols, E., Tayler, J. M and Marks, V. (1972) in: Glucagon (Lefebvre, P. J. and Unger, R. H. eds.) Pergamon Press, Oxford, New York, Toronto, Sydney, Braunschweig p. 151.
[13] Fußgänger, R. D., Goberna, R., Schröder, K. E., Laube, H. and Pfeiffer, E. F. (1971) Diabetologia 7, 480.
[14] Ashcroft, S. J. H., Weerasinghe, L. C. and Randle, P. J. (1973) Biochem. J. 132, 223.
[15] Hellman, B., Idahl, L. A., Lernmark, A., Sehlin, J. and Täljedal, I. B. (1974) Arch. Biochem. Biophys. 162, 448.
[16] Hahn, H. J., Hellman, B., Lernmark, A., Sehlin, J. and Täljedal, I. B. (1974) J. Biol. Chem. 249, 5275.
[17] Hahn, H. J., Lippmann, H. G., Knospe, S., Michael, R. (1970) Acta Biol. Med. Germ. 25, 573.
[18] Speck, G. A., Ziegler, B., Hahn, H. J. (1974) Radiobiol. Radioth. 15, 85.
[19] Ziegler, M., Michael, R., Stein, H. E. and Klatt, D. (1974) Radiobiol. Radioth. 15, 79.