EFFECTS OF D-GLYCERALDEHYDE AND D-GLUCOSE ON THE INSULIN RELEASE OF PANCREATIC ISLETS ISOLATED FROM THE NEWBORN RAT

A. ÅGREN, A. ANDERSSON and C. HELLERSTRÖM
Department of Histology, University of Uppsala, Box 571, S-751 23 Uppsala, Sweden

Received 6 July 1976
Revised version received 28 September 1976

1. Introduction

It seems now well established that in a number of species glucose is a poor stimulus to insulin release in the offspring during the late fetal and immediate neonatal periods [1–5]. Previous attempts to explain the low insulin response as reflecting a deficient accumulation of cyclic AMP in the B-cells were not conclusive [5], and the precise cause of the functional immaturity therefore remains an enigma. We have now tried to evaluate this problem further by comparing the effects of D-glucose with those of D-glyceraldehyde on the insulin release from isolated islets of the developing rat. It has been shown previously that D-glyceraldehyde is a potent secretagogue in the adult rat and that this effect is probably related to its metabolism by the B-cell [6–10]. D-glyceraldehyde enters the glycolytic pathway at the triose phosphate step and would therefore stimulate the insulin secretion independently of the glucose phosphorylation of the B-cell.

We found that in the islets of the newborn rat there was a modest enhancement of the insulin release, when the glucose concentration was raised from zero to 1.4 mM, whereas no further stimulation was recorded above this level. By contrast, D-glyceraldehyde was a potent secretagogue in the islets of the neonatal rats. The observations support the idea that the transition from an immature to an adult insulin response to glucose is associated with changes in the glycolytic pathway prior to the triose phosphate step. This is in agreement with our previous observations of marked alterations in glucose phosphorylation of the B-cell in the neonatal rat [11], and conforms to observations by others suggesting that this enzyme system might be of significance for the regulation of the insulin release [12,13].

Address for correspondence: Dr A. Ågren, Department of Histology, University of Uppsala, Box 571, S-751 23 Uppsala, Sweden

2. Materials and methods

2.1. *Animals and islet isolation*

Sprague-Dawley rats of both sexes were used and all animals were given a commercial pelleted food supplied by Anticimex, Stockholm, Sweden. Newborn rats (litter size 9–16 animals) were kept with their mothers until the experiment began, 6–16 h after birth. Adult controls consisted of 5–8 months old female rats. After decapitation and removal of the pancreas islets were isolated by a collagenase technique [14].

2.2. *Incubations and insulin assay*

Groups of 10 isolated were preincubated for 60 min at 37°C in 250 μl of a bicarbonate buffered medium [15] containing 3.3 mM D-glucose and 2 mg/ml bovine plasma albumine (Fraction V, Armour Pharmaceutical Co. Ltd, Eastbourne, England). The gas phase consisted of 95% O_2: 5% CO_2. Each islet group was subsequently transferred by a braking pipette to another incubation vial and was incubated for 60 min in 250 μl of the same buffer containing

either D-glucose or D-glyceraldehyde (Koch-Light Laboratories Ltd, Colnbrook, Bucks., England) in concentrations given below. In some experiments 10 mM theophylline was added to the medium together with either D-glucose or D-glyceraldehyde. Samples of the medium were frozen immediately after the incubation and stored at −20°C until assay of immunoreactive insulin [16], using kits obtained from the Radiochemical Centre, Amersham, England. Crystalline mouse insulin was used as a standard and the error of a single determination was ± 10%.

2.3. *DNA-assay*

The DNA content of the isolated islets was measured after incubation of the islets in duplicate samples of homogenates in distilled water of either 10 or 20 islets according to the method described by Kissane and Robins [17] and modified by Hinegardner [18]. Calf thymus DNA was used as a standard. The fluorescence was measured in a Farrand Ratio fluorimeter (Farrand Optical Company, Mt Vernon, N.Y., USA) in a final volume of 150 μl.

3. Results

The measurements of islet DNA showed that in the adult rat there was 43.6 ± 3.2 ng DNA per isolated islets (n = 6), whereas the corresponding value for the tiny islets of the newborn animals was only 22.9 ± 1.0 ng per islet (n = 6). In order to make possible relevant comparisons between animal groups of different ages the insulin release data were expressed on the basis of islet DNA content. When studying the dose–response curve of the newborn animals (see below) DNA measurements were, however, not carried out and these results were expressed on the basis of islet number.

The effects of different glucose concentrations on the insulin release of neonatal rat islets are shown in fig.1. In comparison with the islets incubated in the absence of glucose there was a significantly higher insulin release from those incubated at 1.4 mM glucose, whereas no further stimulation was noted by raising the glucose concentration above this level. Table 1 shows a comparison between the insulin release from

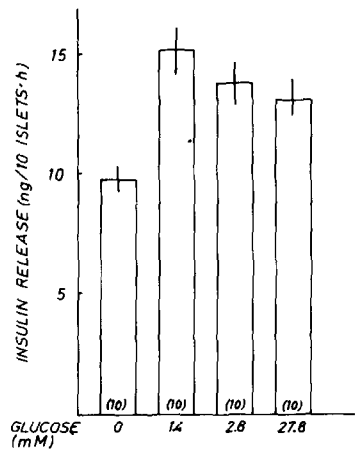

Fig.1. Insulin release of neonatal rat islets exposed to various glucose concentrations. Values are expressed in ng insulin per 10 islets and hour. Mean values ± S.E. Numbers of observations within parentheses.

Table 1
Effects of D-glucose and D-glyceraldehyde on the insulin release from isolated pancreatic islets of neonatal and adult rats

	Insulin release (pg/ng islet DNA · hour)			
Test compound	1-day-old rats		Adult rats	
Glucose, 3.3 mM	39 ± 5	(8)	33 ± 10	(9)
Glucose, 16.7 mM	33 ± 4	(8)	94 ± 8	(9)
Glucose, 16.7 mM + theophylline, 10.0 mM	151 ± 23	(6)	226 ± 13	(7)
D-Glyceraldehyde, 1.0 mM	47 ± 3	(8)	56 ± 15	(9)
D-Glyceraldehyde, 10.0 mM	75 ± 11	(8)	69 ± 9	(9)
D-Glyceraldehyde, 10.0 mM + theophylline, 10.0 mM	144 ± 22	(6)	140 ± 14	(7)

Means ± S.E. Number of adult animals or newborn litters in brackets

neonatal and adult rat islets in response to either D-glucose or D-glyceraldehyde. While an increase of the glucose concentration from 3.3–16.7 mM was without effect on the neonatal islets, there was a significant stimulation ($P < 0.001$) in isolated adult islets. Addition of 10 mM theophylline to the high-glucose medium stimulated the insulin release very markedly from the islets of both newborn and adult animals ($P < 0.001$). In contrast to high concentrations of glucose, 10 mM D-glyceraldehyde elicited a secretory response in the newborn rat islets. This stimulatory action was more pronounced than that observed either with 3.3 mM glucose ($P < 0.001$) or 1 mM D-glyceraldehyde ($P < 0.01$). In adult islets the insulin release in the presence of 10 mM D-glyceraldehyde was higher than that observed with 3.3 mM glucose ($P < 0.001$), but not significantly different from that induced by 1 mM D-glyceraldehyde. There was a marked stimulation of the insulin release in both animal groups when 10 mM of theophylline was combined with 10 mM D-glyceraldehyde.

4. Discussion

The present finding of a small, although significant, insulin response to a very low glucose concentration in the neonatal rat islets indicates a marked alteration of the dose response curve as compared to the adult rat [5,10] and further emphasizes that glucose recognition by the immature B-cell differs from that in the adult. A similar left deviation of the dose response has been reported in the fetal lamb, which also shows a maximal insulin response at 1.4 mM glucose [19]. Although the precise mechanism which governs this type of response cannot be decided at present, it agrees with the view that a putative glucoreceptor is saturated at relatively low glucose concentrations in the neonatal islets. A glucose regulation of this kind may, in fact, be well adapted to the metabolic demand in the perinatal period since the fetal plasma concentration of glucose generally is below the maternal concentration and may fall to very low values in the immediate neonatal period.

In contrast to glucose, D-glyceraldehyde stimulated the insulin release already in the newborn. This observation further confirms a previous report [19] suggesting that the poor insulin response to glucose in the neonate does not merely reflect a sustained inhibitory action of high in vivo levels of catecholamines, since these compounds inhibit the insulin response to D-glyceraldehyde as efficiently as to glucose [7,9,10]. Other studies have also shown that D-glyceraldehyde is readily oxidized by the B-cell and that neither this process nor the insulin releasing action of D-glyceraldehyde are inhibited by mannoheptulose, a compound which is known to inhibit glucose phosphorylation by the B-cell and to block its insulin release response to glucose [6,7,9,10]. The dose response curve describing the secretagogic effect of D-glyceraldehyde nevertheless shows the same sigmoidicity as that of glucose although the minimal effective concentration appears lower [7,8,10]. Furthermore, D-glyceraldehyde inhibits the production of $^{14}CO_2$ from D-[U-^{14}C]-glucose, possibly by dilution of labelled glucose metabolites [7,9]. Altogether these data strongly suggest that the insulin releasing action of D-glyceraldehyde is related to its metabolism by the B-cell and that this compound may produce a metabolic signal, which is located in the glycolytic pathway at or below the triose phosphate step and which may be common to that of glucose [6,7,9,10].

It follows from the above considerations that the transition from an immature to an adult type of insulin response to glucose would involve factors located above the triose phosphate step. Glucose phosphorylation may be of significance in this context, since this process is markedly altered when the insulin response to glucose changes into the adult pattern [11]. In addition, recent studies [20] suggest that induction in vitro of glucose sensitivity in immature B-cells parallels the appearance of a hexokinase with a high K_m value for glucose. Further characterization of the glucose phosphorylating enzymes of the B-cells is, however, required before their precise role in glucose recognition can be evaluated.

Acknowledgements

Financial support from Expressens Prenatalforskningsfond, the Swedish Society for Medical Research, the University of Uppsala and the Swedish Medical Research Council is gratefully acknowledged.

References

[1] Grasso, S., Saporito, N., Messina, A. and Reitano, G. (1968) Lancet 2, 755–756.
[2] Asplund, K., Westman, S. and Hellerström, C. (1969) Diabetologia 5, 262.
[3] Asplund, K. (1972) Diabetologia 8, 13–159.
[4] Milner, R. D. G., Leach, F. N. and Jack, P. M. B. (1975) in: Carbohydrate Metabolism in Pregnancy and the Newborn (Sutherland, H. W. and Stowers, N. M. eds) pp. 83–104, Churchill Livingstone, Edinburgh.
[5] Grill, V., Asplund, K., Hellerström, C. and Cerasi, E. (1975) Diabetes 24, 746–752.
[6] Ashcroft, S. J. H., Weerasinghe, L. C. C. and Randle, P. J. (1973) Biochem. J. 132, 223–231.
[7] Hellman, B., Idahl, L. Å., Lernmark, Å., Sehlin, J. and Täljedal, I. B. (1974) Arch. Biochem. Biophys. 162, 448–457.
[8] Hahn, H. J., Ziegler, M. and Mohr, E. (1974) FEBS Lett. 49, 100–102.
[9] Jain, K., Logothetopoulos, J. and Zucker, P. (1975) Biochim. Biophys. Acta 399, 384–394.
[10] Malaisse, W. J., Herchulez, A., Levy, J., Sener, A., Pipeleers, D. G., Devis, G., Somers, G. and van Obberghen, E. (1976) Mol. Cell Endocr. 4, 1–2.
[11] Andersson, A., Grill, V., Asplund, K., Berne, C., Ågren, A. and Hellerström, C. (1975) in: Early Diabetes in Early Life (Camerini-Davalos, R. and Cole, H. S. eds) pp. 49–56, Academic Press Inc., New York.
[12] Ashcroft, S. J. H. and Randle, P. J. (1970) Biochem. J. 119, 5–15.
[13] Ashcroft, S. J. H., Hedeskov, C. J. and Randle, P. J. (1970) Biochem. J. 118, 143–154.
[14] Asplund, K. and Hellerström, C. (1972) Horm. Metab. Res. 4, 159–163.
[15] Gey, G. O. and Gey, M. K. (1936) Amer. J. Cancer 27, 45–49.
[16] Heding, L. (1972) Diabetologia 8, 260–266.
[17] Kissane, J. M. and Robins, E. (1958) J. Biol. Chem. 223, 184–188.
[18] Hinegardner, R. T. (1971) Anal. Biochem. 39, 197–201.
[19] Shelly, H. J., Bassett, J. M. and Milner, R. D. G. (1975) Brit. Med. Bull. 31, 37–43.
[20] Ågren, A., Brolin S. E. and Hellerström, C. (1975) Diabetologia 11, 329.