

Glucose Stimulation of Insulin Secretion from the Isolated Pancreas of Foetal and Newborn Rats*

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Summary. Pancreatic glands of 22-day-old rat foetuses or 0–12 day old rats were incubated in a Krebs-Ringer phosphate-buffered salt solution, and the insulin secretion measured at a lower (0.6 mg/ml) or a higher (3 mg/ml) glucose concentration in the medium. Stimulation of the insulin release by glucose could not be detected until the second postnatal day, when there was a marked increase in the amount of hormone secreted into the medium. Since the presence of insulin has been demonstrated in the B-cells of rat foetuses, the data suggest that in this species the cellular mechanism for insulin synthesis becomes manifest considerably earlier than that for glucose-regulated insulin release.

Stimulation par le glucose de la sécrétion d'insuline par le pancréas isolé de rats foetaux et nouveau-nés

Résumé. Les glandes pancréatiques de fœtus de rats âgés de 22 jours ou de rats âgés de 0–12 jours ont été incubées dans une solution tampon au phosphate de Krebs Ringer, et la sécrétion d'insuline a été mesurée pour une faible concentration de glucose (0.6 mg/ml) ou pour une concentration de glucose plus élevée (3 mg/ml) dans le milieu. La stimulation de la libération d'insuline par le glucose ne pouvait pas être décelée jusqu'au deuxième jour postnatal, où il y avait une nette augmentation de la quantité d'hormone sécrétée dans le milieu.

Puisque la présence d'insuline a été démontrée dans les cellules B de fœtus de rats, les données suggèrent que dans cette espèce le mécanisme cellulaire de la synthèse de l'insuline devient manifeste beaucoup plus tôt que celui de la libération d'insuline régulée par le glucose.

Stimulation der Insulinsekretion mit Glucose aus dem isolierten Pankreas von foetalen und neugeborenen Ratten

Zusammenfassung. Das Pankreas von 22 Tage alten Rattenfoeten oder 0–12 Tage alten Ratten wurde in einer Krebs-Ringer-Phosphat-Puffer-Lösung inkubiert, und die Insulin-Sekretion bei einer niedrigeren (0.6 mg/ml) oder höheren (3 mg/ml) Glucosekonzentration der Lösung gemessen. Stimulation der Insulinfreisetzung durch Glucose konnte bis zum 2. postnatalen Tag nicht festgestellt werden, an dem ein bemerkenswerter Anstieg des in die Lösung sezernierten Hormons eintrat. Da die Gegenwart von Insulin in den B-Zellen von Rattenfoeten gezeigt wurde, lassen die Werte vermuten, daß sich bei dieser Spezies der Zellmechanismus für Insulin-Synthese beträchtlich früher manifestiert als die glucoseregulierte Insulinfreisetzung.

Key-words: Insulin secretion, isolated pancreas, pancreas of foetal rats.

Introduction

There is much evidence to indicate that synthesis of insulin occurs in the pancreatic B-cells at a rather early stage of mammalian foetal development. Morphological studies in man have demonstrated storage of the hormone as cytoplasmic secretion granules already at a foetal age of about 10–12 weeks [2, 3, 12], and insulin is present in the B-cells in foetuses of the rat, guinea-pig, sheep and mouse before the last third of the gestational period [1, 8, 10, 17, 19, 20]. The correlation between the morphological maturation of the B-cell and the development of the mechanisms for regulation of insulin release is, however, poorly understood. An approach to this question has been made in the present study by measuring the insulin release from foetal and neonatal rat pancreas incubated in media with different glucose concentrations.

Materials and Methods

Foetal and neonatal Sprague-Dawley rats of both sexes were used. The foetal age was estimated with

an accuracy of ± 5 h, and the post-partum age was determined on the basis of witnessed delivery. The neonatal rats remained with their mothers until the start of the experiment.

After decapitation of the animal the pancreas was quickly excised, rinsed in incubation medium, blotted on filter paper, weighed and suspended in ice-cold medium. The subsequent incubations were performed mainly as described by COORE and RANDLE [4]. The incubation medium consisted of Krebs-Ringer phosphate-buffered salt solution [21] supplemented with bovine serum albumin, 2 mg/ml (Fraction V; Armour Pharmaceutical Co. Ltd., Eastbourne, England), together with pyruvate, glutamate and fumarate each at a concentration of 5 mM [4]. Glucose was added to the medium at a concentration of 0.6 or 3.0 mg/ml.

The pancreatic glands (10–20 mg wet weight) were incubated without slicing at $+37^{\circ}\text{C}$ in Warburg vessels with constant shaking. The gas phase consisted of pure oxygen. After preincubation for half an hour at the lower glucose concentration, the glands were rinsed in fresh medium and incubated for a further 30 min in new vessels containing 0.5 ml of medium with the same glucose concentration. This was followed by a final incubation period of the same length at the higher glucose concentration. Samples of the media from the

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two last incubation periods were immediately frozen for subsequent insulin determinations according to HALES and RANDLE [11]. Crystalline mouse insulin (approx. 23 IU/mg) was used as a standard. Antibody and ^{125}I -insulin were obtained from The Radiochemical Centre, Amersham, England. Duplicate assays were carried out, the error of a single determination being $\pm 12\%$. Absolute rates of insulin release have been expressed as ng/mg wet weight per 30 min.

Results

As shown in Table 1 the rate of insulin release at the lower glucose concentration displayed small vari-

Table 1. Mean insulin secretion (\pm S.E.M.) from incubated pancreatic glands of foetal and newborn rats. The significance of the difference between the secretion rates at lower and higher glucose concentrations in the incubation media is also shown

Age Days	Number of observations (Number of litters)	Insulin secretion (ng/mg pancreas/30 min)		P
		0.6 mg glucose/ml	3.0 mg glucose/ml	
$-\frac{1}{2}$	7 (3)	1.5 ± 0.3	1.5 ± 0.3	> 0.05
$\frac{1}{2}$	8 (3)	1.5 ± 0.3	1.1 ± 0.3	> 0.05
1	6 (3)	1.8 ± 0.7	0.7 ± 0.2	> 0.05
2	11 (2)	1.4 ± 0.3	3.8 ± 0.8	< 0.05
3	11 (2)	1.7 ± 0.2	5.6 ± 0.9	< 0.001
5	6 (2)	1.4 ± 0.3	3.9 ± 0.3	< 0.01
6	9 (3)	1.7 ± 0.3	4.1 ± 0.9	< 0.05
12	7 (1)	1.1 ± 0.3	3.0 ± 0.3	< 0.001

ations between animals of different postnatal ages. This basal rate of insulin secretion was also of the same order as that observed just before birth. A stimulation of the insulin release by glucose could not be detected until the second postnatal day, when in most experiments there was a very marked increase in the amount of hormone secreted into the medium. It is worthy of note, however, that also on the second postnatal day 4 out of 11 glands remained unaffected by glucose. In the older animals only occasional specimens showed this lack of response. The great majority of pancreatic glands at these ages contained B-cells displaying a 2- to 6-fold increase of the insulin release during incubation with the higher glucose concentration.

Discussion

The present data indicate that stimulation *in vitro* of insulin release from the rat pancreas by glucose does not occur until the second postnatal day. Previous studies with morphological, histochemical and biochemical methods have conclusively demonstrated the presence of insulin in the B-cells of rats at about the 18th intrauterine day [5, 6, 8, 18, 20]. It seems probable, therefore, that the cellular mechanism for insulin synthesis becomes manifest considerably earlier than that for glucose regulation of insulin release. There is,

however, some evidence to suggest that the insulin storage of the B-cells is considerably raised by glucose administration to one-day-old rats [15]. Glucose might therefore enhance the synthesis of insulin before it becomes able to stimulate the release of this hormone.

The rate of morphological maturation of mammalian B-cells differs a great deal between various species. Whereas distinct secretion granules can be seen in the light microscope in man very early during the foetal life [2, 3, 12], they have not been detected until the second half of gestation in the rat. It seems probable that corresponding differences exist in the development of the mechanism for the regulation of insulin release. In fact, recent data have shown that in the human newborn, glucose administration through the umbilical cord increased the blood level of immunoreactive insulin already a few hours after birth [13]. This increase was, however, much slower than that reported to occur upon intravenous administration of glucose in adult life and also in newborns of diabetic mothers [13]. The latter observation suggests that maternal diabetes may accelerate the functional maturation of the foetal B-cell.

Since insulin has been found not to penetrate the placental membranes of rats [2a, 7], the possible demands for this hormone must be met by the foetal endocrine pancreas itself. It is, however, still obscure to what extent the foetus depends on a supply of insulin for its normal development. Insulin sensitivity (in terms of a facilitated glucose entry across cellular membranes) seems to develop gradually during intrauterine life [9]. Further studies are required to evaluate, to what extent insulin is present in the foetal blood circulation before the B-cell can respond to glucose, and what mechanisms are responsible for its secretion. In recent studies VECCHIO et al. [22] and LAMBERT et al. [14] have shown that insulin secretion from the cultured foetal pancreas of rats may be stimulated by a number of factors other than glucose. In addition, MILNER and WRIGHT [16] reported a prompt rise in the plasma insulin level after administration of glucagon in one-day-old human infants.

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