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Glucose stimulates the biosynthesis of rat I and II insulin to an equal extent in isolated pancreatic islets

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The effects of glucose on insulin biosynthesis were studied by measuring the incorporation of radiolabelled amino acids into proinsulin/insulin in isolated rat islets. The islets were pulse labelled for 15 min with [^{3}H]leucine (present in rat insulin I and II) or [^{35}S]methionine (unique to rat insulin II) and then incubated for a 165 min post-label (chase) period during which the majority of labelled proinsulin was converted to insulin but under conditions whereby >95% of radiolabelled proinsulin or insulin was retained in the islets. The newly synthesized, labelled, insulin was analyzed by high performance liquid chromatography. Rat I and II insulin biosynthesis was stimulated by 16.7 mM glucose to the same extent.

Proinsulin synthesis; Insulin; Secretagogue stimulation; HPLC; (Islets of Langerhans, Rat)

1. INTRODUCTION

Insulin is produced in the B-cells of pancreatic islets from its precursor preproinsulin in a series of coordinated biochemical and subcellular events [1]. Its biosynthesis can be rapidly and specifically stimulated by glucose and certain other secretagogues [2–4].

Unlike the majority of mammals, not one but two different insulins are present in the pancreas of rats [5,6] and mice [7,8]. In the rat, these two insulins are encoded for by two nonallelic genes [9,10] with the corresponding mRNAs showing about 93% homology [11,12]. The primary translation products, rat preproinsulins I and II, differ by 3 amino acid substitutions in the signal peptide sequence, 2 in the C-peptide region and 2 in the B-chain [9,12]. Mouse insulins I and II are identical in sequence to those of the rat [7,8].

The biosynthesis of rat insulins I and II has been studied previously [6,13,14]. However, the issue of whether glucose regulates the synthesis of both rat insulins I and II, although addressed, has remained unresolved due to inadequate resolution of rat insulin I from proinsulins and the non-quantitative transfer to nitrocellulose of rat insulin II with the immunoelectrophoretic system used [15–17]. To avoid such analytical problems, we have used high-performance liquid chromatography (HPLC) to monitor glucose stimulation of rat insulin I and II biosynthesis in isolated islets.

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2. EXPERIMENTAL

Pancreatic islets were isolated from adult male CD rats by collagenase digestion [18]. Isolated islets were then maintained in tissue culture (RPMI 1640 medium containing 8.3 mM glucose and 10% (v/v) newborn calf serum) for at least 18 h to recover from the isolation procedure.

In order to monitor rat insulin biosynthesis, groups of 100 islets were pulse-labelled in 250 μl modified Krebs-Ringer bicarbonate buffer containing 10 mM Hepes and 0.1% (w/v) bovine serum albumin, pH 7.4 (KRB-Hepes), in the

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presence of 0.25 mCi L-[4,5-^{3}H]leucine or L-[^{35}S]methionine, at a basal (2.8 mM) or stimulatory (16.7 mM) glucose concentration for 15 min, at 37°C. The islets were then washed in ice-cold, unlabelled KRB-Hepes containing 2.8 mM glucose and incubated for a 165 min post-label chase period at 37°C in 3 ml RPMI 1640 containing 2.8 mM glucose and 10% (v/v) newborn calf serum. The islets were then removed, washed in cold phosphate-buffered saline containing 0.1% (w/v) bovine serum albumin, pH 7.4 (PBS-BSA), suspended in 100 μl PBS-BSA in 1.5 ml microfuge tubes, and stored at −20°C pending analysis.

Islets suspended in 100 μl PBS-BSA were sonicated (25 W, 10 s), and the sonicated debris pelleted by 1 min centrifugation (10000 × g) in a microfuge. Aliquots (10 μl) of this supernatant were then used to analyze radiolabelled amino acid incorporated into rat insulin I and II. Quantitative analysis of rat insulin I and II using HPLC was performed as described in [19]. In brief, the reversed-phase HPLC system is a C-18 column eluted isocratically with 35% acetonitrile:H_2O (9:1) and 65% 50 mM phosphoric acid, 20 mM triethylamine, 50 mM sodium perchlorate adjusted to pH 3.0 with NaOH, at a flow rate of 1 ml/min.

Rat insulins I and II both contain 6 leucine residues. Rat I insulin contains no methionine, but there is a substitution of a lysine for a methionine at residue 29 of the rat insulin II B-chain [2,15]. The use of [^{35}S]methionine instead of [^{3}H]leucine in the pulse-labelling period therefore allows for monitoring of only rat insulin II biosynthesis.

3. RESULTS

An HPLC elution profile of a representative aliquot of sonicated rat islets taken after a 15 min pulse label with [^{3}H]leucine and a 165 min chase, is shown in fig.1. Radioactivity eluting from the HPLC system was found associated with both rat insulin I (eluting at 30–35 min) and rat insulin II (eluting at 38–43 min). These two peaks of radioactivity associated with insulin coincided with two peaks of UV absorbance at 213 nm. When [^{35}S]methionine was used in place of [^{3}H]leucine for pulse labelling, radioactivity was only detected in the insulin II peak in accordance with the known primary sequence of the two rat insulins [2,15].

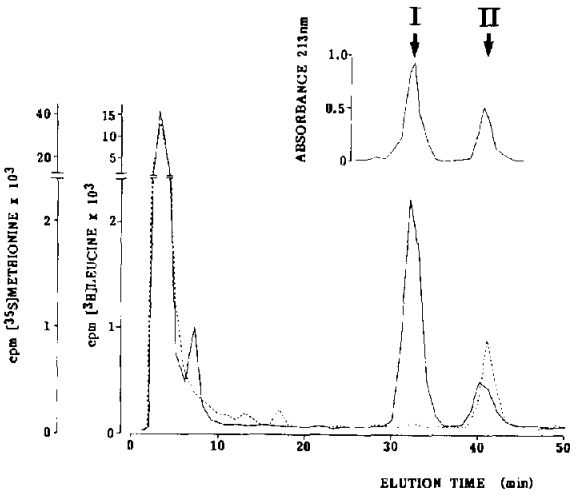

Fig.1. HPLC elution profile for the separation of rat insulins I and II following pulse labelling of islets for 15 min with [^{3}H]leucine (——) or [^{35}S]methionine (···) and a 165 min chase. The known elution times for the two insulins are shown by the numbered arrows. The inset shows UV absorbance ($A_{213} \times 10^{-3}$) for the HPLC analysis of the sample which had been labelled with [^{3}H]leucine.

There was always more insulin I than insulin II whether measured in terms of [^{3}H]leucine incorporation (reflecting newly synthesized insulins) or UV absorbance at 213 nm (reflecting total islet insulin content).

Although we [19] and others [20] have described methods for the separation of rat insulins I and II by HPLC, such is not the case for the two rat proinsulins. Here, it was therefore necessary to ensure that essentially all labelled proinsulin had been converted to insulin before analysis. This was achieved by incubating the pulse-labelled islets for 165 min at 2.8 mM glucose. It has been shown previously that >95% of newly synthesized proinsulin is converted to insulin within 180 min [2,19,21]. Furthermore, by selecting a low glucose concentration (2.8 mM) for the chase incubation, it was possible to ensure that the majority of the labelled insulin was retained in the islets rather than being released [21]. Under such conditions, the measurement of radioactivity in rat insulins I and II can therefore be used as an index of their respective rates of synthesis. These data are shown in table 1. The radioactivity in insulin I or II for

Table 1

Glucose stimulation of the incorporation of radiolabelled amino acids into rat insulins I and II

Rat insulin	Radiolabelled amino acid	Radioactivity in insulin (cpm/ng)		Fold-stimulation
	Glucose (mM)	2.8	16.7	16.7 vs 2.8
I	[^{3}H]leucine	40.0 ± 3.1	81.6 ± 8.2	2.43 ± 0.11
II		10.4 ± 0.6	26.7 ± 3.4	2.61 ± 0.38
II	[^{35}S]methionine	6.3 ± 1.0	16.7 ± 2.3	2.45 ± 0.16

Islets were pulse-labelled with the given amino acid for 15 min at either 2.8 or 16.7 mM glucose. The islets were then incubated for a 165 min chase period without radioactivity at 2.8 mM glucose. Radioactivity in insulins I or II was assessed by HPLC and the data (means ± SE, n = 3–5 separate experiments) expressed as cpm/ng total islet insulin immunoreactivity (measured by radioimmunoassay prior to HPLC). Note that there is no methionine in rat insulin I

each experiment has been normalized to the total islet insulin content (i.e. the combined immunoreactivity of insulins I and II) as measured by radioimmunoassay [22] before HPLC analysis. In agreement with the HPLC profile shown in fig.1, there was always more radioactivity in insulin I than II. Raising glucose from 2.8 to 16.7 mM was found to stimulate the labelling of both insulin I and II to the same extent, and the data obtained for insulin II using [^{3}H]leucine were similar to those using [^{35}S]methionine. The ratio of radioactivity in insulin I relative to that in insulin II, however, was the same following a pulse label with [^{3}H]leucine at either 2.8 or 16.7 mM glucose.

4. DISCUSSION

The specific and rapid stimulation of proinsulin/insulin biosynthesis by elevated extracellular glucose concentrations has been known for over 20 years [2–4,23]. The short-term (<2 h) regulation of insulin biosynthesis appears to be independent of net preproinsulin mRNA synthesis and thus arises at a translational level [24–27]. There appears to be a shift of cytoplasmic free preproinsulin mRNA to membrane-bound polysomes leading to increased synthesis of proinsulin upon glucose stimulation [25,26]. Exposure to glucose for >2 h, however, has been shown to result in increased preproinsulin mRNA synthesis. In the present study, isolated rat islets were only subjected to short-term glucose stimulation (15 min) and glucose stimulation of synthesis was therefore limited to the translational effects.

The glucose regulation of the biosynthesis of rat insulins I and II in isolated islets has been studied previously [15], and it was suggested that synthesis of insulin I was stimulated to a greater extent than that of rat insulin II. It has since become apparent that these conclusions were incorrect, since there were inherent problems in interpreting the data due to inadequate separation and/or non-quantitative recovery of rat insulins and proinsulins by the electrophoretic system used [17]. In this study, we have depended upon a separation system previously shown [19] to (i) be quantitative, and (ii) resolve rat insulins I and II. Our data indicate unequivocally that 16.7 mM glucose stimulates rat insulin I and II biosynthesis to an equal extent (about 2.5-fold).

Rat I and II preproinsulin mRNA levels have been measured in rat islets [31] and insulinoma cells [30,32] and shown to be present in approximately equal amounts. It has, furthermore, been shown that both rat preproinsulin mRNAs appear to be regulated coordinately in vivo following fasting and refeeding of rats with sucrose [31]. In the present study there was more rat I than II insulin in islets, whether measured by radioactivity after biolabelling with [^{3}H]leucine or by UV ab-

sorption (and radioimmunoassay; not shown) following HPLC analysis. High levels of rat I relative to II insulin have been found by others in both native [6,13,14] and transformed [30] B-cells. The discrepancy between the data for the mRNA levels compared with those of the native insulins remains to be explained but must presumably reflect differential rates of protein turnover and/or different translation efficiency for the two mRNA species. Regardless of the final explanation, our data confirm that insulin biosynthesis can be stimulated by glucose during a 15 min pulse-labelling period. This effect is presumed to be due to stimulation of translation, and occurs to an equal extent for both rat insulins.

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