RNA SYNTHESIS AND THE STIMULATION OF INSULIN BIOSYNTHESIS BY GLUCOSE

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1. Introduction

Glucose stimulates both incorporation of amino acids into insulin [1, 2] and incoproration of label from orotic acid into RNA of rat islets of Langerhans [3]. We have shown that glucose, in the physiological concentration range, stimulates incorporation of amino acids into a proinsulin-containing protein fraction from isolated rat islets with a considerable degree of specificity and have interpreted these results as indicating increased proinsulin biosynthesis in response to glucose [4].

In this report we describe experiments in which actinomycin D was used to investigate the relationship between RNA synthesis and the stimulation of proinsulin synthesis by glucose. Actinomycin D, added before glucose, inhibited amino acid incorporation but a stimulatory effect of glucose was still present, suggesting that the glucose stimulation of proinsulin synthesis is not mediated by newly synthesized RNA. Actinomycin D, added after the glucose stimulation, caused an additional stimulation of protein synthesis. Actinomycin D reduced the rate of secretion of insulin when added before glucose but had no immediate effect when added after glucose. The possibility that the drug is affecting other processes directly, as well as RNA synthesis, is considered.

2. Materials and methods

Actinomycin D was a gift from Merck, Sharp and Dohme, Rahway, New Jersey. Collagenase was obtained from Worthington through Cambrian Chemicals, Croydon, Surrey. 5-^{3}H-Uridine (27.6 Ci/mmole), ^{14}C-L-leucine (311 mCi/mmole) and insulin immunoassay kits were obtained from the Radiochemical Centre, Amersham, Bucks.

Islets of Langerhans were prepared by collagenase digestion [5] of pancreata from male Sprague-Dawley rats weighing 350–500 g. Incubation in bicarbonate-buffered medium, isolation of a proinsulin fraction by Sephadex G-50 chromatography of labelled islet proteins and immunoassay of insulin have been described previously [4].

3. Results

Proteins from the Islets of Langerhans can be separated into two major peaks on Sephadex G-50 [4] one of which, fraction P, contains proinsulin. Fig. 1 shows the effect on the Sephadex G-50 profile of labelled islet proteins of increasing the glucose concentration in the medium from 2 mM to 20 mM. The proportion of radioactivity in fraction P is increased from about 6% to about 30%. It has been argued [4] that this glucose effect represents a stimulation of the synthesis of proinsulin. The average recovery of radioactivity from the column in 24 experiments was 99%.

Actinomycin D concentrations of 1 and 5 μg/ml inhibit incorporation of ^{3}H-uridine into total islet RNA by 91% and 96% respectively (data not shown). 99% of the ^{3}H-uridine incorporation is sensitive to ribonuclease. Under our conditions, uridine is a better precursor than orotic acid.

Table 1 shows the effect of actinomycin D under various conditions on the incorporation of ^{14}C-leucine

Table 1
The effect of actinomycin D on protein synthesis in islets.

Expt. no.	Glucose concn. (mM)	Actinomycin D concn. (μg/ml)	Incubation time (min)	cpm eluted before fraction P.	cpm in fraction P.	% in fraction P of total cpm	% change of cpm in fraction P by actinomycin D
(a) Islets preincubated 15–30 min with actinomycin D before the glucose stimulus							
1	2	–	75	624 ± 165 (2)	39 ± 4 (2)	6.1 ± 0.8 (2)	
	2	5	75	624 ± 44 (2)	45 ± 4 (2)	6.7 ± 4.0 (2)	0
	20	–	75	2165 ± 360 (2)	848 ± 320 (2)	28.0 ± 4.0 (2)	−69
	20	5	75	698 ± 70 (2)	260 ± 40 (2)	27.0 ± 1.0 (2)	
2	20	–	75	2618 ± 440 (4)	1203 ± 216 (4)	31.4 ± 0.3 (4)	−44
	20	5	75	1507 ± 230 (3)	673 ± 100 (3)	30.9 ± 0.6 (3)	
3	20	–	75	1835 ± 170 (3)	746 ± 117 (3)	28.6 ± 1.4 (3)	−59
	20	1	75	840 ± 279 (3)	304 ± 103 (3)	27.0 ± 3.0 (3)	
(b) Both actinomycin D and glucose stimulus at time zero							
4	20	–	30	974 ± 75 (3)	164 ± 14 (3)	14.4 ± 0.8 (3)	−23
	20	1	30	667 ± 13 (3)	127 ± 7 (3)	16.0 ± 0.5 (3)	
(c) 75 min preincubation with 20 mM glucose before actinomycin D							
5	20	–	30	1965 ± 278 (3)	828 ± 80 (3)	29.9 ± 1.3 (3)	+24
	20	1	30	2456 ± 110 (3)	1018 ± 98 (3)	29.2 ± 1.0 (3)	
6	20	–	30	4562 ± 193 (5)	1625 ± 151 (5)	26.1 ± 1.3 (5)	+21
	20	1	30	5168 ± 188 (5)	1962 ± 104 (5)		

Results are expressed as cpm per incubation tube. Within an experiment each tube contained the same number of islets. Between experiments the number of islets per tube varied from 15 to 20. After incubation, islets were resuspended in 0.5 ml of medium containing ^{14}C-leucine (4 μCi/ml) and glucose and actinomycin D at the appropriate concentrations and incubated at 37° in an atmosphere of 95% O_2, 5% CO_2. Protein fractions were isolated as previously described [4] and radioactivity was assayed using a Packard liquid scintillation spectrometer.

into fraction P protein (fig. 1) and into the remainder of the labelled protein eluted from the Sephadex G-50 column. The stimulatory effect of glucose above the 2 mM threshold level on the labelling of protein is clearly seen. The drug had no effect on incorporation at sub-threshold levels (2 mM; [4]) of glucose. When the islets were exposed to the drug before the glucose simulus, considerable inhibition of incorporation occurred, but an effect of glucose on labelling of the proinsulin fraction was still observed and the proportion in fraction P of the total incorporation was unchanged by the drug.

Actinomycin D added after stimulation by glucose caused an additional stimulation of amino acid incorporation (table 1c). In no case was there any preferential effect of the drug on the labelling of fraction P relative to total protein labelling.

Fig. 2 shows the effect of actinomycin D, added either before or after the glucose stimulus, on insulin secretion. When added before the glucose stimulus, it reduced the rate of secretion in response to glucose. Added afterwards, it had no immediate effect on the glucose-stimulated secretion rate.

4. Discussion

Although actinomycin D is known to have inhibitory effects on processes other than RNA synthesis [6, 7] the observation (table 1a,b) that glucose stimulated incorporation into the proinsulin fraction even when RNA synthesis was inhibited strongly suggests that the effect of glucose is not mediated by newly synthesized RNA. The stimulation of incorpora-

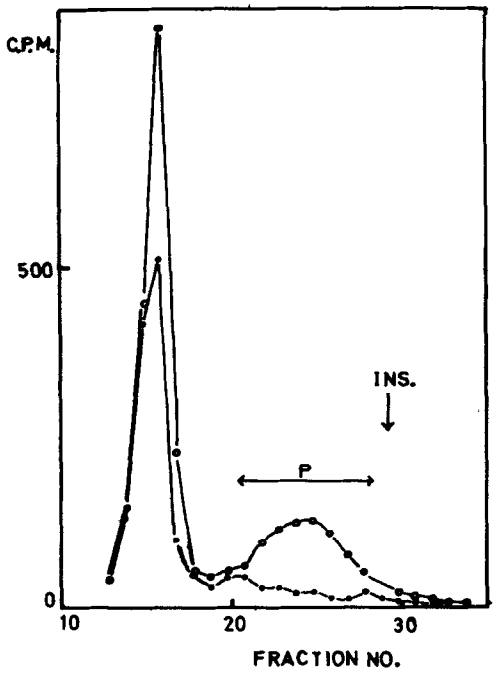

Fig. 1. The effect of glucose on Sephadex G-50 elution profiles of radioactive islet proteins. 25 Islets were incubated for 2 hr with ^{14}C-leucine (4 μCi/ml) and either 2 mM (●—●) or 20 mM (○—○) glucose. The proinsulin fraction (P) and the peak position of an ox insulin marker (INS) are indicated.

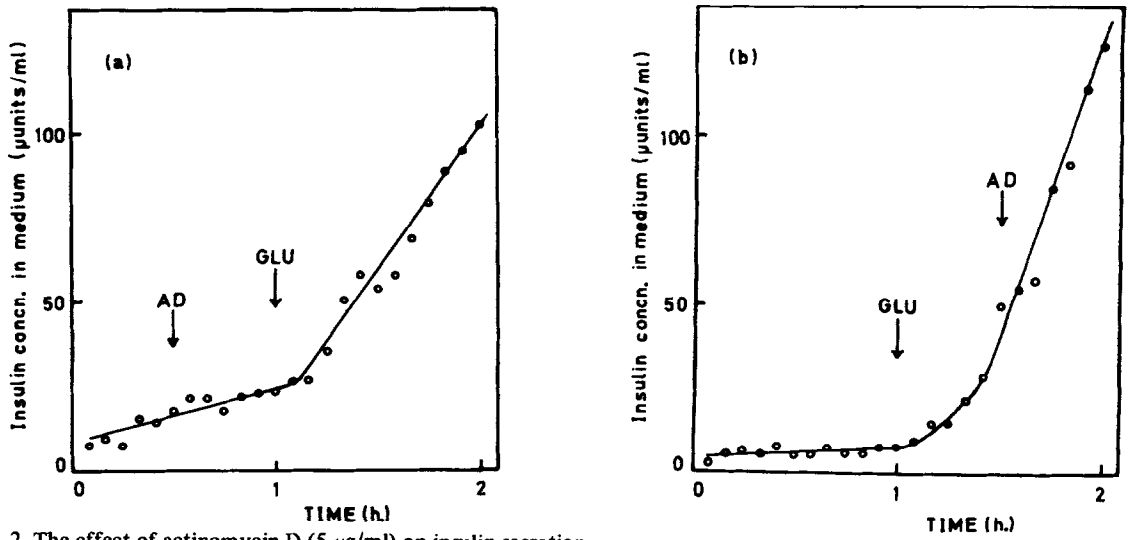

Fig. 2. The effect of actinomycin D (5 μg/ml) on insulin secretion.

(a) 25 islets were incubated at 37° in 40 ml of medium containing 2 mM glucose and 2 mg/ml bovine serum albumin. Duplicate samples (0.1 ml) were taken for immunoassay and replaced by an equal volume of fresh medium. After 60 min the glucose concentration was increased to 20 mM and after 90 min actinomycin D was added. Corrections to the estimates of insulin secretion were made for sampling and volume changes after additions. Ox insulin was used as a standard in the immununoassay for rat insulin and the figures given are ox insulin equivalents (25 units = 1 mg of ox insulin).

(b) Details as in (a), except that actinomycin D was added after 30 min.

tion into fraction P by glucose is reduced, but not abolished, by the drug and the proportion in fraction P of the total radioactivity incorporated remains at the stimulated level. These experiments do not exclude the possibility that preformed RNA is released from the nucleus in response to glucose. The results of table 1b suggest that the increased rate of amino acid incorporation into protein in response to glucose does not reach its maximal level rapidly.

The data in table 1c show that when protein synthesis has been stimulated by glucose, addition of 1 μg/ml of actinomycin D caused a small but consistent further stimulation of protein synthesis. The effect was not specific for the proinsulin fraction. Small stimulations of amino acid incorporation by the drug have also been observed in cod islets [8] and sarcoma-37 ascites cells in the presence of glucose [7].

This effect of actinomycin D is difficult to explain in terms of inhibition of RNA synthesis and is reminiscent of the superinduction phenomenon reported by Tomkins and his associates in HTC cells induced by glucocorticoids [9]. The actinomycin effect in our experiments was, however, not specific for proinsulin. Because of this we are reluctant to explain our results in terms of the hypothesis put forward by these workers [9].

The experiments on insulin secretion suggest that actinomycin D may be affecting processes other than RNA and protein synthesis. When the drug is added before the glucose stimulus, the response of the secretion rate to glucose is halved (fig. 2a). Cycloheximide, however, does not affect the secretion rate in response to glucose until about 45 min after stimulation [4], even when protein synthesis has been inhibited by cycloheximide for an hour before stimulation [10]. The effect of actinomycin D on insulin secretion is, therefore, not a consequence of its effects on protein synthesis. No inhibitory effect of actinomycin on secretion was observed when it was added after the glucose stimulus.

The inhibition of protein synthesis and insulin secretion may be caused, at least partly, by effects of actinomycin on glucose metabolism, since protein synthesis by unstimulated islets is unaffected by the drug and it seems unlikely that insulin secretion is closely associated with RNA synthesis. It has previously been suggested that the drug may have such effects [6].

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