EFFECT OF GLUCOSE AND TOLBUTAMIDE ON RNA SYNTHESIS IN ISOLATED ISLETS OF LANGERHANS FROM RAT PANCREAS

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1. Introduction

Glucose and tolbutamide, both potent stimulators of the secretion of insulin from the islets of Langerhans, have different effects on the synthesis of new hormone. While in the presence of high glucose concentrations the synthesis is increased, it is diminished in the presence of tolbutamide [1, 2]. The mode of how these substances affect the biosynthesis of insulin is unknown. A relevant explanation could be that glucose and tolbutamide have a different effect on RNA synthesis of the islet cells. We therefore analysed the incorporation of ^{3}H-uridine into RNA fractions of these cells under various concentrations of glucose and tolbutamide.

2. Methods

The islets of Langerhans were isolated from rat pancreas according to the method of Gerner et al. [3]. The incubation was performed in closed polystyrene tubes in Hanks' balanced salt solution [4] with 0.5% bovine albumin (Fluka AG) and in the presence of various concentrations of glucose, tolbutamide and actinomycin D; the final vol of the incubation mixture was 0.5 ml.

In the experiments where RNA synthesis was analyzed, each tube contained 25 islets, 10 nmoles adenosine, guanosine and cytosine and 5 μCi ^{3}H-uridine (5 Ci/mM, New England Nuclear); in those where protein synthesis was followed, 10 islets together with 1.25 μCi ^{3}H-leucine (17 Ci/mM, Radiochemical Centre, Amersham) and 10 nmoles of the other 19 L-amino acids. The incubation was performed for 1 hr at 37°.

After the incubation period the tubes were chilled in an ice-water bath. In the experiments where the incorporation of ^{3}H-uridine was analyzed, 10 ml of cold Hanks' solution were added, and the content of each tube transferred to small Petri dishes. Duplicates of ten islets were selected from each Petri dish and replaced into two new tubes. After the addition of 0.5 mg of soluble RNA from yeast (Boehringer Mannheim) the volume was adjusted to 1 ml with Hanks' solution. RNA was precipitated and washed twice with cold TCA (final conc. 5%). The resulting pellet was dissolved in 1 ml of 1 M NH_4OH and after standing overnight at room temp radioactivity was determined in a liquid scintillation spectrometer. An additional lysis of the cells with 2% Triton X 100 and 1% SDS before RNA precipitation was carried out to make sure that unincorporated ^{3}H-uridine and low molecular weight products were completely separated. The lysis had no influence on the results and was therefore omitted.

In the experiments where protein synthesis was analyzed, protein was precipitated and washed 4 times with 10% TCA.

3. Results

Table 1 demonstrates that the incorporation of ^{3}H-uridine into the TCA precipitable RNA fraction of isolated islets of Langerhans from rat pancreas is nearly doubled when the glucose concentration is raised from 0.5 to 3.0 mg/ml incubation medium (exp. 3). Glucose stimulated RNA synthesis is inhibited up to 80% in the presence of 10 μg actinomycin D/ml (exp. 3). Addition of 1 mg tolbutamide/ml at low glucose concentration clearly inhibits RNA syn-

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Table 1
Effect of glucose, tolbutamide and actinomycin D on the incorporation of ^{3}H-uridine into the RNA of isolated islets of Langerhans from rat pancreas.

Experiment	Glucose (mg/ml)	Tolbutamide (mg/ml)	Actinomycin D (μg/ml)	Ipm/ 10 islets	n	Incorporation of ^{3}H-uridine (%)	
1	0.5	0	0	701 ± 63	8	100 ± 9	
	0.5	1	0	452 ± 34	8	64 ± 5	
2	3.0	0	0	535 ± 70	6	100 ± 13	
	3.0	1	0	504 ± 61	8	94 ± 11	
3	0.5	0	0	383 ± 31	7	100 ± 8	
	3.0	0	0	769 ± 124	7	200 ± 32	100 ± 16
	3.0	0	10	130 ± 15	7		17 ± 2

For details, see Methods; n = number of determinations.

thesis (exp. 1). An inhibitory effect of tolbutamide, however, can not be detected at a concentration of 3.0 mg glucose/ml (exp. 2).

When protein synthesis is analyzed, tolbutamide inhibits the incorporation of ^{3}H-leucine into the TCA-precipitable protein fraction of the cells to about 50% at 0.5, as well as at 3.0, mg glucose/ml (table 2). Glucose alone shows a strong stimulation of leucine incorporation [1, 2, 5].

4. Discussion

The experiments described above show that the rate of RNA synthesis in isolated islets of Langerhans from the rat is affected by the concentration of glucose in the incubation medium (table 1, exp. 3). Similar results have been obtained by Permut and Kipnis [6] and Jarrett et al. [7]. Whether the increased RNA production in the presence of 3.0 mg glucose/ml, where insulin biosynthesis is strongly stimulated [1, 2, 5], reflects the synthesis of new messenger RNA for this hormone must still be questionable. Experiments of Morris and Korner [8] suggested that the induction of insulin biosynthesis by glucose does not depend on the synthesis of specific messenger RNA; total protein synthesis and insulin biosynthesis were affected in a same manner when the cells were incubated with glucose in the presence of actinomycin D. Another explanation of the glucose effect on RNA synthesis is the induction of ribosomal RNA synthesis, which will be investigated in further experiments.

The observed inhibition of RNA synthesis by tolbutamide in the presence of 0.5 mg glucose/ml could be the result of a lower uptake of ^{3}H-uridine into the cells or a direct effect of tolbutamide on RNA biosynthesis. Hellman [9] has shown that several hypoglycemic sulfonylureas decrease the ATP level of islet cells, whereas it is increased by glucose. It is therefore possible that either the uptake of ^{3}H-uridine into the cells or the incorporation into RNA is mediated by this alteration of the ATP level. The finding that tolbutamide has no significant effect on the incorporation of ^{3}H-uridine in the presence of 3.0 mg glucose/ml, can then be explained by the opposite effect of glucose and tolbutamide on the ATP level in the cells. But this explanation does not answer the question

Table 2
Effect of glucose and tolbutamide on the incorporation of ^{3}H-leucine into the TCA precipitable protein of isolated islets of Langerhans from rat pancreas.

Glucose (mg/ml)	Tolbutamide (mg/ml)	Ipm/ 10 islets	n	Incorporation of ^{3}H-leucine (%)	
0.5	0	298 ± 39	8	100 ± 14	
0.5	1	166 ± 50	6	56 ± 17	
3.0	0	922 ± 78	8	309 ± 27	100 ± 8
3.0	1	486 ± 93	4		53 ± 10

For details, see Methods; n = number of determinations.

why protein biosynthesis is inhibited by tolbutamide at both glucose concentrations.

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