INSULIN BIOSYNTHESIS AFTER INHIBITION OF THE TRYPTIC ACTIVITY OF PANCREATIC TISSUE IN RATS

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Insulin biosynthesis in rat pancreatic tissue homogenate was investigated after total suppression of tryptic activity in order to inhibit the proteolytic conversion of proinsulin into insulin. The gland tissue was preincubated with soy trypsin inhibitor for 2 h at 37°C. The tissue was then incubated in the presence of glycine-1-C^{14} for a further 30 and 60 min. Similar samples not containing the inhibitor were used as the control. Insulin extracted from the tissue was purified by repeated recrystallization followed by running through Sephadex G-50. The radioactivity was determined in eluates of fractions corresponding to the insulin peak. Inhibition of tryptic activity was found not to inhibit insulin formation. Insulin synthesis presumably takes place not only by proteolytic transformation of proinsulin into insulin, but also from other precursors and, in particular, from presynthesized A- and B-chains.

KEY WORDS: insulin biosynthesis, tryptic activity; pancreas.

Investigations have shown that insulin synthesis takes place from separately formed A- and B-chains [1, 3, 4, 7]. The view has recently been put forward [2, 10-14] that insulin is formed only from a single-chain precursor, known as proinsulin, by a mechanism of proteolytic conversion [5, 6, 11, 15]. However, it is not yet clear which enzyme systems participate in this process and there is no evidence that such a process takes place in vivo. If insulin is formed only from proinsulin (and, as it is considered, under the influence of trypsin or of trypsin-like enzymes), complete inhibition of the tryptic activity of the pancreatic tissue should sharply inhibit the formation of the hormone. If, however, insulin synthesis is effected from separately formed chains, its synthesis should continue even after 100% inhibition of trypsin-like activity.

To study this problem the rate of incorporation of an amino acid into insulin was compared in the presence and absence of trypsin inhibitor.

EXPERIMENTAL METHOD

A pancreatic tissue homogenate weighing 0.5 g in 2.5 ml Ringer–Krebs buffer (pH 7.4) was preincubated with 1.25 mg soy trypsin inhibitor (VEB Arzneimittelwerk, East Germany) and without it for 1-2 h at 37°C, with slow, steady agitation. After centrifuging at 6,000 rpm and filtration through Perlon gauze the proteolytic activity of the tissue was determined using N-α-benzoyl-L-arginine-amide (Reanal, Hungary) and the substrate [9]: 0.4 ml of filtrate was incubated with 0.4 ml of a 0.01 M solution of the substrate for 2 h. The sample was titrated with 0.01 N HCl solution. The results of the titration were expressed in percentages of hydrolysis, assuming that 0.01 ml acid is equivalent to 1% hydrolysis. After preincubation with the inhibitor for 2 h, 100% inhibition of trypsin-like activity was obtained. In the next experiments 3 g of the pancreatic tissue homogenate was preincubated in 15 ml Ringer–Krebs buffer (pH 7.4) with or with-

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TABLE 1. Incorporation of Glycine-1-C^{14} into Insulin in the Presence and Absence of Trypsin Inhibitor (counts/min/mg)

Conditions of incubation	Eluate from first column		Eluate from second column	
	after incubation of samples with glycine-1-C^{14}			
	30 min	60 min	30 min	60 min
Without inhibitor	105	336		
	110	308	145	290
With inhibitor	145	385		
	171	357	157	341

out 7.5 mg of inhibitor at 37°C, with steady agitation. All the samples were then treated with 1 μCi glycine-1-C^{14} (specific activity 100 μCi/mg) and incubated for a further 30 and 60 min under the same conditions. At the end of incubation the bottle was removed by centrifuging and the tissue was washed 3 times to remove free radioactivity with 50-ml portions of buffer containing a hundredfold excess of unlabeled glycine. The tissue of each sample was then frozen with dry ice and the insulin was extracted with alcohol acidified to pH 2.0. The preparation was recrystallized three times with the aid of nonradioactive crystalline bovine insulin and purified twice on a column (1 × 50 cm) with Sephadex G-50, using 50% acetic acid as the eluent. Eluates of fractions belonging to the insulin peak were pooled and evaporated to dryness, quantitatively estimated [8], and their radioactivity measured on a gas-flow counter. The results were expressed in counts/min/mg protein.

EXPERIMENTAL RESULTS

It is clear from the data in Table 1 that the radioactivity of insulin was similar in samples incubated with and without the inhibitor. The specific activity of the insulin showed no significant change after additional purification on a second column. After incubation for 60 min the radioactivity of the insulin was twice as high as after incubation for 30 min. However, after 30 min the specific radioactivity of the insulin was already relatively high despite the considerable dilution of the radioactivity with unlabeled insulin.

Suppression of tryptic activity thus did not inhibit insulin biosynthesis. Although other proteolytic enzymes [15] than trypsin (or enzymes possessing the specificity of trypsin) participate in the process of transformation of proinsulin into insulin, as the supporters of this hypothesis insist, nevertheless the complete suppression of tryptic activity should have been reflected in the intensity of insulin biosynthesis had it been formed entirely from proinsulin. Consequently, it can be concluded from these findings that insulin formation takes place from other precursors and, in particular, as the writers showed previously [1], from free A- and B-chains previously synthesized separately.

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