EFFECT OF ADRENALECTOMY AND PARTIAL RESECTION OF THE PANCREAS ON INSULIN RESYNTHESIS IN THE LIVER

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Investigation of incorporation of labeled amino acids into insulin in the liver and pancreas showed that the ratios between specific activities of A and B chains of insulin in these organs differ considerably. These ratios are modified by disturbance of islet-cell function. It is postulated that the A and B chains of insulin, which are formed in the pancreas, undergo considerable modification in the liver, and that particular stages of the formation of peptide portions of the chains take place in the liver.

Insulin reaching the liver from the pancreas via the bloodstream undergoes marked degradation there because of the presence of an insulinase (insulin-glutathione transdehydrogenase) in the liver tissue [5, 8, 14, 16-18, 20]. This enzyme reduces disulfide bonds in insulin with the formation of free A and B chains. Its action is reversible; in the presence of the oxidized form of glutathione, biologically active hormone is resynthesized from the chains thus formed [6]. It is also assumed that the chains are subjected to the action of various proteases, leading to considerable degradation of their structure [9, 10]. However, there is little information concerning possible pathways of resynthesis of this protein in the liver.

With these facts in mind, an investigation using radioactive amino acids was undertaken to study the pathways of insulin resynthesis in the liver.

EXPERIMENTAL METHOD

Since the conversions of insulin in the liver are closely linked with its biosynthesis in the pancreas, to obtain a more complete picture of conversions of the A and B chains it was decided to study the metabolism of insulin isolated from the liver in animals with modified islet-cell function. For this purpose two experimental models were selected: resection of the tail of the pancreas and adrenalectomy.

Rats undergoing resection of one-third of the splenic portion of the pancreas were used in the experiment two weeks after the operation, and adrenalectomized rats 24 h after the operation, and during these periods the animals received glucose-saline.

Male rats of all groups, including the control, weighing 150-200 g, were injected intraperitoneally with one of the following labeled amino acids: 1-C^{14}-tyrosine, S^{35}-cysteine, and 1-C^{14}-glycine, with specific activities of 58, 48, and 130 μCi/mg, respectively, in doses of 200 μCi/100 g body weight.

The animals were decapitated 2 h later. Insulin was isolated from the pancreas and liver of 4-5 animals by extraction in acid ethanol [11], followed by repeated reprecipitation at the isoelectric point of insulin [19]. In some experiments, insulin was extracted from both tissues by means of anti-insulin guinea pig serum [2, 15], while in other experiments the insulin was purified on a column with Sephadex G-50 [3].

Insulin from both tissues, isolated and purified by one of the methods described above, was separated into A and B chains [4, 7, 12].

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TABLE 1. Ratios between Specific Activities of A and B chains of Insulin Isolated from Pancreas and Liver (4 experiments)

Radioactive amino acid	Group of animals: intact			with resectioned pancreas			adrenalectomized		
	pancreas	liver	P	pancreas	liver	P	pancreas	liver	P
C^{14}-tyrosine	0,30	1,09	<0,05	0,15	0,30	<0,05	0,60	0,40	<0,02
S^{35}-cysteine	0,17	0,34	<0,02						
C^{14}-glycine	0,10	0,30	<0,05						

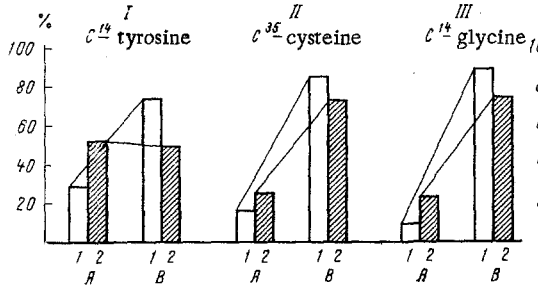

Fig. 1

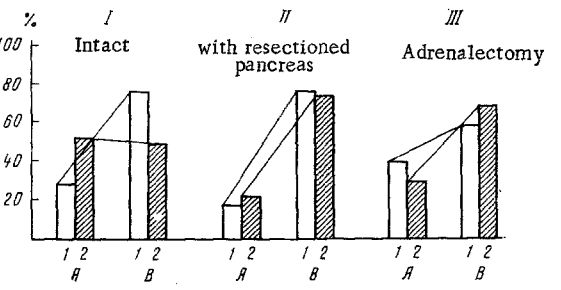

Fig. 2

Fig. 1. Incorporation of labeled amino acids into A and B chains of insulin in liver and pancreas of intact rats. Ordinate, relative values of specific activity of chains (in %); sum of A and B chains taken as 100%; I) injection of C^{14}-tyrosine into rats; II) injection of S^{35}-cysteine; III) injection of C^{14}-glycine; 1) pancreas; 2) liver.

Fig. 2. Incorporation of C^{14}-tyrosine into A and B chains of insulin in liver and pancreas after resection of tail of pancreas and adrenalectomy. I) intact rats; II) animals undergoing resection of the pancreas; III) adrenalectomized animals. Remainder of legend as in Fig. 1.

EXPERIMENTAL RESULTS

Specific activities of A and B chains of insulin from the liver of intact animals differed in their incorporation of all radioactive amino acids used from the corresponding chains from the pancreas (Fig. 1).

The ratios between specific activities of the A and B chains in the liver were high in value for incorporation of each amino acid used, and differed variously from the ratios in the pancreas (Table 1). This is clearly reflected by changes in the gradient of the lines connecting the A and B chains of the same tissue (Fig. 1).

After these changes in adrenal and pancreatic function, these ratios characteristically diverged sharply, even for incorporation of the same amino acid (Fig. 2, Table 1). Incorporation of C^{14}-tyrosine into the A chain of liver insulin fell sharply after adrenalectomy, and more sharply still after resection of the pancreas, while incorporation of C^{14}-tyrosine into the A chain of pancreatic insulin fell only after resection of the pancreas, and after adrenalectomy it rose above the level in intact animals. Incorporation of C^{14}-tyrosine into the B chain in the liver showed little change, whereas incorporation of this amino acid into the B chain in the pancreas fell below its level in intact animals in both pathological states.

If resynthesis of insulin in the liver consisted of nothing more than recombination of the A and B chains formed as a result of the breakdown of insulin brought from the pancreas, the ratio between the specific activities of the chains in both tissues during utilization of different amino acids would be identical. However, these ratios differed considerably, and this could be evidence of active metabolism of the insulin chains in the liver. The hypothesis of considerable degradation of A and B chains after insulin breakdown in the liver and their subsequent resynthesis into the whole insulin molecule is also supported by the fact

TABLE 2. Specific Activities of Amino-Acid Residues of A and B Chains of Insulin Isolated from Liver of Intact Animals (mean data for 12-15 animals in group)*

Amino acid	A chain	B chain	A/B
C^{14}-tyrosine	7 641±501,0	7 200±515,0	1,1
C^{35}-cysteine	1 926±108,3	5 400±318,7	0,17
C^{14}-glycine	1 701±95,1	1 886±107,5	0,9
C^{14}-serine *	1 225±80,9	6 643±437,1	0,1

*Since this amino acid is converted into serine in experiments with C^{14}-glycine [1, 12], radioactivity belonging to the glycine and serine residues was determined in insulin (and its chains) isolated from both tissues. For this purpose, insulin and its B chain were subjected to total acid hydrolysis, followed by radiochromatographic analysis of the hydrolysates. The radioactivity of the glycine and serine residues in the A chain was calculated.

that the ratios between radioactivities of the amino-acid residues in the liver of intact animals during utilization of each of the four labeled amino acids varied within wide limits (Table 2).

As Table 2 shows, the ratios between these values varied between 0.1 and 1.1, reflecting differences in the level of metabolic activity of individual segments of each chain.

These amino-acid residues are located in different parts of the polypeptide A and B chains of insulin, and the radioactive amino acids used consequently reflect metabolism of the insulin chains throughout their extent (for the A chain from 1 to 20 amino-acid residues, and for the B chain from 7 to 26). The variability thus obtained for ratios between the values of specific activities during comparison of the two chains for utilization of each labeled amino acid could therefore reflect differences in the character of metabolism of peptide segments of the chains by transformation either of individual peptide segments or of individual amino acids.

This hypothesis is also supported by facts indicating differences in incorporation of C^{14}-tyrosine into the A and B chains of insulin in the liver in association with disturbances of islet-cell function, which do not correlate with changes in incorporation of this amino acid into the A and B chains in the pancreas, and, indeed, are sometimes opposite in direction (Fig. 2).

It can therefore be assumed that the A and B chains of insulin, which are formed in the pancreas and are precursors of insulin in the liver, undergo considerable transformations in the liver, and that incorporation of amino acids takes place into certain peptide segments. Modification of islet-cell function is accompnaied by changes in metabolism of the insulin polypeptide chains in the liver, as shown by the discovery of changes in metabolism of liver insulin when the synthesis of insulin by the pancreas is disturbed. Consequently, active breakdown and resynthesis of insulin takes place in the liver tissue.

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