INSULIN PRECURSORS IN HORMONE FORMATION AFTER RESECTION OF THE DISTAL PART OF THE PANCREAS

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The isotope label dilution method was used to investigate the utilization of the polypeptide chains of insulin and of dipeptides in hormone formation after resection of the distal part of the pancreas in rats. It was shown that although the free chains are utilized in insulin formation in the resected organ, the process is less marked than in the intact gland. Arg-Gly and Gly-Ser dipeptides stimulated insulin biosynthesis.

In the early stages after resection of the distal part of the pancreas in rats [1] insulin biosynthesis is considerably accelerated. Not only amino acids, but also the A- and B-polypeptide chains of insulin and low-molecular-weight peptides act as precursors of insulin in intact animals [2, 3]. Presumably the acceleration of insulin synthesis after resection of the pancreas is linked with the preferential use of ready-made "blocks," i.e., peptide fragments, in its biosynthesis.

The utilization of polypetide chains and dipeptides in insulin formation after resection of the pancreas was investigated.

EXPERIMENTAL METHOD

Synthetic dipeptides Arg-Gly and Gly-Ser (Reanal) and A- and B-chains of insulin, obtained by Varandani's method [4], were used.

Glycine-1-C^{14} (0.3 mCi/100 g body weight, specific activity 130 μCi/mg) was injected intraperitoneally into rats on the seventh day after resection of the distal part of the pancreas and also intact animals. The choice of labeled amino acid was determined by the fact that its unlabeled amino-acid residue is a component of the "ballast" dipeptides used and of both chains of insulin. The animals were decapitated 24 h later, after the excretion of most of the free labeled amino acid. Minced pancreatic tissue (3 g) was incubated for 1 h at 37°C in 15 ml Ringer–Krebs buffer, pH 7.4, with 10 mg of "ballast" A- and B-chains of insulin separately. Control samples did not contain the "ballast."

The experiments with the dipeptides were carried out under similar conditions; Arg-Gly or Gly-Ser were added to the samples as "ballast." Both dipeptides corresponded to fragments contained only in the B-chain of insulin.

After incubation insulin was isolated from each sample [5] and divided into its A- and B-chains [6]. The radioactivity of the chains was expressed in pulses per minute per μmole amino-acid residue.

A decrease in the radioactivity of only the homonymous insulin chains during incubation with A- and B-chains, and also a decrease in radioactivity of only the B-chain on incubation with the dipeptides reflected utilization of the peptide fragments during insulin synthesis.

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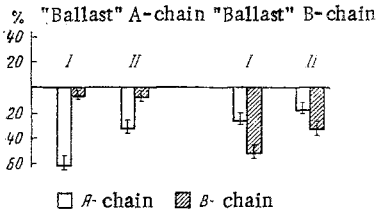

Fig. 1

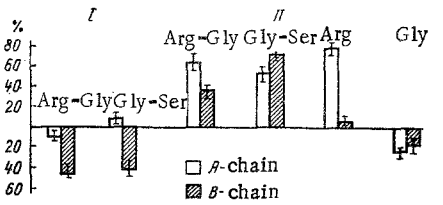

Fig. 2

Fig. 1. Change in radioactivity of A- and B-chains of insulin isolated from pancreas of intact (I) and partially pancreatectomized (II) rats under the influence of "ballast" chains (% of results obtained without "ballast"). Values given represent M±m.

Fig. 2. Effect of "ballast" dipeptides and amino acids on radioactivity of insulin A- and B-chains, previously labeled with glycine-C^{14}, isolated from pancreas of intact (I) and partially pancreatectomized (II) rats (% of data obtained without "ballast"). Values given represent M±m.

EXPERIMENTAL RESULTS

As the results given in Fig. 1 show, on the addition of "ballast" chains to the gland tissue from the pancreatectomized animals a decrease in the radioactivity predominantly of the homonymous chains of insulin was observed. However, this decrease was less than in the intact animals. Consequently, the utilization of the free chains in insulin formation in animals after resection of the pancreas was less marked than in intact animals. The decrease in radioactivity of the A-chain on incubation of the pancreatic tissue with the B-chain, observed in the animals of both groups, was evidently the result of the partial breakdown of the B-chain.

As the results in Fig. 2 show, on the addition of "ballast" dipeptides to the tissue of the resected gland there was a marked increase in the radioactivity of both chains of insulin, whereas in the intact animals the utilization of dipeptides for insulin synthesis was clearly revealed, as shown by the decrease in radioactivity of the insulin B-chain. The increase in radioactivity of the insulin chains after addition of "ballast" dipeptides to the tissue of the gland taken from the animal (after reaching the maximum of glycine-C^{24} incorporation into insulin in vivo) could be the result of the utilization of peptide fragments with high radioactivity, similar in structure to the fragments of insulin, in the synthesis of this hormone. These fragments may be formed by the breakdown of other proteins accumulating more radioactivity than insulin. A similar phenomenon was observed during the investigation of protein biosynthesis by the regenerating liver [7]. However, against this background of the action of dipeptides in stimulating insulin synthesis it was impossible to judge whether they are utilized directly in the synthesis of the hormone after resection of the pancreas.

Since the stimulant could be due to products of dipeptide breakdown (their amino-acid components), the effect of "ballast" glycine and arginine on insulin biosynthesis was investigated. The experimental conditions were the same as in the experiments with "ballast" dipeptides. The "ballast" glycine did not stimulate formation of the hormone; arginine, however, stimulated synthesis of the A-chain of insulin (Fig. 2). Characteristically the action of these dipeptides in stimulating insulin biosynthesis was selective and was not exhibited on the biosynthesis of total pancreatic proteins.

The acceleration of insulin biosynthesis after resection of the distal part of the pancrease is thus evidently a result of the stimulation of its formation by low-molecular-weight products of protein breakdown.

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