Clearance of Porcine Insulin, Proinsulin, and Connecting Peptide by the Isolated Rat Liver[1] (34589)

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After intravenous injections, we have found the immunological half-life of purified porcine proinsulin to be more prolonged than purified porcine single-component insulin in both swine (22 and 9 min, respectively) and in baboons (18 and 8 min, respectively). Studies in humans have also indicated a longer half-life of proinsulin than insulin (1). These findings have prompted the present investigation of the *in vitro* degradation of insulin, proinsulin, and the connecting peptide that links the A and B chains of insulin in the proinsulin molecule, using an isolated liver perfusion system.

Methods. Intact livers, weighing 4.5–6.8 g, from male Wistar rats, fasted for 48 hr, were cyclically perfused by the method of Hems *et al.* (2). The perfusion fluid consisted of washed human erythrocytes, 2 g/100 ml bovine serum albumin (Cohn, Fraction V), and Krebs-Henseleit buffer, pH 7.4, with a hemoglobin concentration of approximately 3 g/100 ml. After complete isolation from the circulation, the livers were perfused *in situ* by adjusting the hydrostatic pressure of the perfusate to give maximal perfusion rates without liver swelling. This was approximately 2–3 ml/g wet weight of liver. The total perfusion volume was 150 ml at the beginning of the experiment. After a 40-min equilibration period, purified porcine single-component insulin (Lilly), proinsulin (Lilly), or connecting peptide (glutamyl peptide B_{33}–B_{63}) (Lilly) (3) were added to separate perfusates to give final concentrations of 3–15 mμg/ml. Four-milliliter aliquots were removed immediately prior to the addition of the above and every 5 min thereafter for 1 hr. The plasma was immediately separated and frozen until assayed. Viability of the livers was determined by appearance and a lactate/pyruvate ratio of less than 10 (4).

Proinsulin and insulin immunoassays were performed by the cellulose method (5) using porcine proinsulin and insulin iodinated with ^{125}I by the chloramine-T method (6). Ten millimicrograms of single-component insulin were added per 50 lambda of a 1:40,000 dilution of the porcine proinsulin antibody, at least 24 hr prior to immunoassay, to bind antibodies which crossreacted with insulin ("coated" proinsulin antibody) (7). Porcine single-component insulin, proinsulin, and connecting peptide were used as standards. Tracer degradation and antibody excess controls were determined for each test.

Results. In Fig. 1 is shown the percentage binding of the proinsulin–^{125}I tracer by the "coated" proinsulin antibody in the presence of single-component insulin, proinsulin, and connecting peptide. More than 20 mμg of insulin must be present in the assay before it decreases the percentage of tracer bound. The connecting peptide reacted three times as well as proinsulin based on weight or equally on a molar basis. With the insulin antibody used in this study, it can be calculated that proinsulin was 37% as reactive as insulin (Fig. 2). Forty millimicrograms of connecting peptide reacted less than 2% in the insulin immunoassay.

Presented in Fig. 3 are the percentage

[1] This investigation was supported by Grants AM 02456 and AM 05020, National Institute of Arthritis and Metabolic Diseases, United States Public Health Service.

[2] Recipient of United States Public Health Service Special Fellowship 5F3-AM37, 833-02.

[3] Recipient of United States Public Health Service Special Fellowship 2-FO3-AM-38, 491-02.

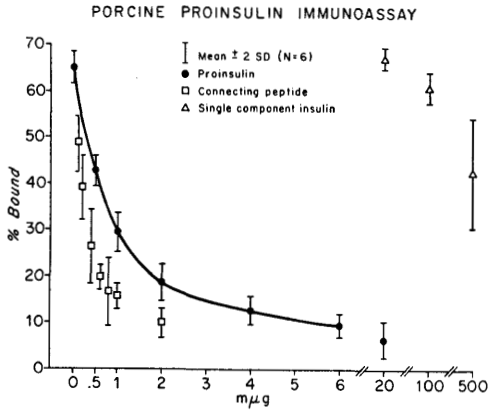

FIG. 1. Standard curves of porcine proinsulin, connecting peptide, and single-component insulin using "coated" proinsulin antibody and proinsulin-^{125}I tracer.

changes in the concentration of insulin, proinsulin, and connecting peptide in the perfusate, arbitrarily taking the 5-min value as 100 %. The $t_{½}$ of insulin in this system was 17 min (least squares calculation). The mean hepatic clearance (k) of insulin was 5.1 ml/min, and the $t_{½}$ of insulin calculated from the hepatic clearance (8) was 16 min, in good agreement with the graphic calculation. In contrast, there was no statistically significant change in the concentration of either proinsulin or the connecting peptide segment during the perfusion period of 1 hr. Furthermore, using the insulin immunoassay, we were unable to demonstrate any conversion of proinsulin to insulin.

Discussion. The hepatic clearance of insulin of 5.1 ml/min was greater than values previously reported and may reflect differences in perfusion methodology (8–10). However, we were unable to demonstrate either a clearance of proinsulin or connecting peptide. If conversion of proinsulin to insulin and connecting peptide had occurred, one would have expected the insulin released to have a similar half-life as when it was perfused alone. However, when the proinsulin perfusion studies were measured in the insulin immunoassay, there was no evidence of hepatic clearance.

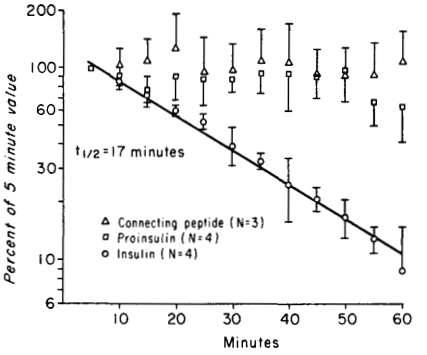

FIG. 3. Percentage changes (mean ± SD) of porcine connecting peptide, proinsulin, and insulin during 1-hr liver perfusion. The immunoreactivity at 5 min was arbitrarily assigned as 100 %.

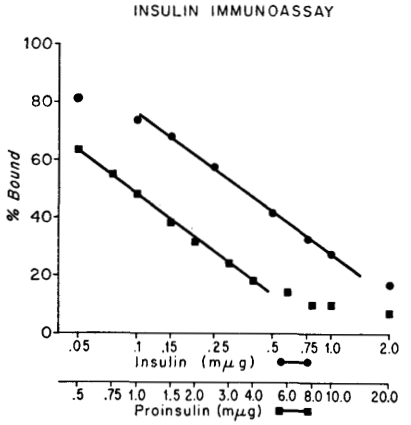

FIG. 2. Standard curves of porcine proinsulin and single-component insulin using an insulin antibody and insulin-^{125}I tracer.

It is of interest to speculate upon the significance of these findings. An initial event in the hepatic degradation of insulin has been considered to be the reductive cleavage of the disulfide bonds between the A and B chains of insulin which is catalyzed by the enzyme glutathione insulin transhydrogenase (11). The finding that proinsulin is capable of spontaneous reoxidation after reduction, with restoration of its immunoreactivity (12) may indicate that this enzyme would be ineffectual in degrading proinsulin. Also, because of the species variability of the connecting peptide segment of the proinsulin molecule (13), its uptake and/or degradation by the liver may show greater species specificity than insulin. However, our find-

ings suggest that tissues other than the liver are responsible for the clearance of proinsulin and this, in part, may account for the prolonged half-life of proinsulin relative to insulin *in vivo*.

Summary. In the isolated rat liver perfusion system, the immunological half-life of crystalline single-component insulin was 17 min. In contrast, there was no significant clearance of either proinsulin or the connecting peptide that links the A and B chain of insulin in the proinsulin molecule. There was also no evidence of conversion of proinsulin to insulin. This may, in part, explain the prolonged *in vivo* half-life of proinsulin relative to insulin.

The authors are most grateful to Dr. M. A. Root (Lilly) for the proinsulin antibody, and to Dr. R. E. Chance (Lilly) for the purified proinsulin, connecting peptide, and single-component insulin used in this study. We also appreciate the excellent technical assistance of Mrs. Carla Gullickson, Miss Ellen Laschansky, and Mrs. Jeanette Teague.

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Received Nov. 15, 1969. P.S.E.B.M., 1970, Vol. 133.