Biological Activity of Synthetic Human Insulin

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Summary. The biological activity of human insulin, prepared by total chemical synthesis, was compared in various tests *in vivo* and *in vitro* with that of natural human or pork insulin. The potencies of the synthetic and natural hormone, as determined by the mouse convulsion assay, by hypoglycaemic effect and diaphragm glycogen increase in fasted rats *in vivo*, or by stimulation of ^{14}C -glucose metabolism in fat cells and binding to anti-insulin serum *in vitro*, did not differ significantly. It is concluded that this synthetic human insulin is biologically equivalent to the natural hormone.

Key words: Synthetic human insulin, mouse convulsion assay, hypoglycaemic effect, diaphragm glycogen, isolated fat cells, anti-insulin serum binding.

The total synthesis of human insulin by a novel fragment condensation procedure has been recently accomplished [1]. This approach, involving directed formation of the three disulfide bonds at different stages of the synthesis, was facilitated by the application of new methods for selective protection of thiol and amino groups [2, 3]. The hormone synthesized in this way has been analyzed by a variety of physico-chemical methods and found to be identical with natural human insulin [1]. The studies presented here characterize the biological activity of the synthetic hormone, prepared by Sieber et al. [1], in comparison with natural insulin extracted from human and porcine pancreas.

Materials and Methods

Synthetic human insulin was prepared as described [1]. Natural human insulin was isolated according to

Romans [4] from pancreas obtained at autopsy. It was highly purified by gel filtration, isoelectric focussing and countercurrent distribution [5]. A sample of the fourth international standard for insulin was generously provided by the WHO International Laboratory for Biological Standards [6]. Porcine insulin was obtained in recrystallized form from Schwarz-Mann, Orangeburg, U. S. A., or as Act-rapid® MC 40 from Novo Industri A/S, Copenhagen.

Stock solutions of these insulins were prepared in saline (0.154 mol/l) acidified to pH 4. Dilutions for *in vitro* experiments were made immediately before use in the appropriate buffer containing bovine or human serum albumin.

D-1- ^{14}C -glucose (spec. activity 2–4 mCi/mmol, code no. CFA. 72) and iodinated ^{125}I -insulin (code no. IM. 38) were purchased from The Radiochemical Centre, Amersham, Great Britain. Antibovine insulin serum from guinea pigs (code no. 64–101, lot no. 21) was obtained from Miles Laboratories, Kankakee, Illinois, U. S. A.

Mice (Tif: MAGf (SPF)) and rats (Tif: RAIf (SPF)) from Tierfarm Sisseln AG, were used at body weights of 25–35 g and 170–200 g, respectively.

Mouse convulsion assays were performed according to [7, 8]. These procedures were modified by using three doses each of test and reference compound, administered SC, to groups of 15 mice fasted for 10 h and kept at 31–33°C during the experiment. The potencies were calculated by quantal response assay (probit analysis) [7, 8].

The hypoglycaemic effect was determined in rats after a fast of 24 h. Blood glucose was assayed by the glucose oxidase/peroxidase method after SC injection of insulin. The potency of synthetic human insulin relative to highly purified pork insulin (Act-

Fig. 1a and b. Effect of human insulin on diaphragm glycogen a and blood glucose b in the fasted rat, determined 90 min after injection. Each point with the vertical bar indicates the mean $\pm$ SEM of ten animals

rapid® MC 40, Novo) was calculated by a six point parallel assay in the dose range 7.5–30 $\mu\text{g/kg}$, using the area under the blood glucose curve as response. Six rats were used for each dose.

The effect on muscle glycogen was studied in the diaphragm of rats fasted for 24 h. Ninety minutes after SC injection of insulin, the rats were sacrificed under sodium pentobarbitone (40 mg/kg IP) anaesthesia. Diaphragm glycogen was extracted with hot potassium hydroxide solution and assayed according to [9]. Blood glucose was determined in trunk blood as described above.

Stimulation of 1- ^{14}C -glucose metabolism (oxidation to $^{14}\text{CO}_2$ and incorporation of ^{14}C into lipids) in rat epididymal fat cells was measured in vitro essentially as described by Rodbell [10]. The concentration required to elicit 50% of the maximal effect (EC_{50}) was determined graphically from dose-response curves covering the range 0.02–13 ng insulin/ml.

The synthetic insulin was tested in competition with ^{125}I -insulin in its binding to anti-bovine insulin serum from guinea pigs under the experimental conditions used in the radioimmunoassay of insulin. The dextran-coated charcoal method of Herbert et al.

[11] was modified by extending the incubation period to 3 days at 4°C. Anti-bovine insulin serum was used at a final dilution of 1:800 000.

Statistical comparison between test and reference compound was made with Student's t-test, the difference being considered significant when $p < 0.05$.

Results

Mouse Convulsion Assay

The potency of synthetic human insulin in the mouse convulsion assay was determined in six separate assays using the fourth international standard for insulin (24.0 IU/mg) as reference. The value calculated from the pooled data was 24.1 IU/mg (95% confidence limits 22.0–25.4).

Hypoglycaemic Effect in the Rat

The time course of the hypoglycaemic effect of synthetic human insulin and porcine insulin in the fasted rat was identical. The potencies of the two insulins, as determined by six-point assay, did not differ significantly.

Muscle Glycogen Increase and Hypoglycaemic Effect in the Rat

Subcutaneous injection of synthetic and natural human insulin into fasted rats caused a dose-dependent increase of diaphragm glycogen and a concomitant decrease of blood glucose, as shown in Fig. 1. Over the dose range 20 to 120 $\mu\text{g/kg}$, no significant difference was observed between synthetic and natural human insulin.

Stimulation of ^{14}C -Glucose Metabolism in Fat Cells

The stimulation by synthetic human insulin (concentration range 0.02–13 ng/ml) of 1- ^{14}C -glucose oxidation to $^{14}\text{CO}_2$ and of incorporation of ^{14}C from glucose into the lipid fraction from fat cells followed a typical sigmoid curve.

The concentrations required to elicit half of the maximal effect (EC_{50}) for synthetic human, natural human and natural porcine insulin, respectively, were 0.38 ± 0.061 , 0.41 ± 0.023 and 0.37 ± 0.051 ng/ml for glucose oxidation and 0.40 ± 0.075 , 0.45 ± 0.024 and 0.38 ± 0.073 ng/ml for ^{14}C -incorporation into lipid (3 expts. each, mean $\pm$ SEM).

None of the differences between the three insulins was statistically significant at the 5% level.

Binding to Anti Insulin Serum

Binding to an anti-bovine insulin serum from guinea pigs was measured by the conventional radioimmunoassay technique. No significant difference between synthetic and natural human insulin and natural porcine insulin was observed in the concentration range 0.2–3.2 ng/ml.

Discussion

Some biological activities of insulin extracted from human pancreas have been described previously. Two groups of investigators compared human with porcine insulin in the radioimmunoassay and found similar binding activity [12, 13]. In addition, comparable activities were also noted with respect to other parameters, i. e. hypoglycaemic effect in rabbits [14], oxidation of ^{14}C -glucose in rat adipose tissue [12], and ^{14}C -glucose incorporation into glycogen of adipose tissue and diaphragm in the rat [12, 13]. Furthermore, a semisynthetic human insulin was studied recently and found to exhibit a biological activity of 26–28 IU/mg, determined by lowering the blood sugar in rabbits [15].

The purpose of the studies reported here was to compare the biological activity of human insulin obtained through a novel, total chemical synthesis [1] with that of the natural hormone.

This comparison was based on five different tests, comprising characteristic effects of insulin both in vivo and in vitro. The results, presented above, reveal no significant difference between test and reference insulin in all variables determined. It is therefore concluded, that the synthetic human insulin studied has full biological activity and is thus equivalent to the hormone of natural origin.

Acknowledgements. We are greatly indebted to Drs. B. Kamber, A. Hartmann, A. Jöhl, B. Riniker, P. Sieber, and W. Rittel for generous supply of human insulin. The technical assistance of Miss J. Motz, Miss F. Arnold, Mrs. L. Hartmann, Mr. R. Lindecker, and Mr. R. Schwaller is thankfully acknowledged.

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Received: December 7, 1976, and in revised form: March 3, 1977

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