Diabetologia (1984) 27: 414–415

Diabetologia

Rapid communications

Human proinsulin standards

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Summary. Two new batches of pancreatic human proinsulin have been compared with biosynthetic human proinsulin. Standards of these three proinsulin preparations were made on the basis of quantitative amino-acid analyses and compared in two proinsulin radioimmunoassays with a proinsulin standard prepared 14 years ago. The curves of the new standards were superimposable. However, they differed considerably from the curve of the old standard which proved to be only one-third of the strength of the new standards, thereby leading to a threefold over-estimation of proinsulin concentrations when the old standard is used. We conclude that the new standards should replace previously used standards.

Key words: Human proinsulin, radioimmunoassay, standards.

Radioimmunoassays for the measurement of circulating human proinsulin have been developed [1–3]. However, their use has been limited to a few laboratories due to shortage of a well-defined human proinsulin standard. Until recently, a pancreatic human proinsulin standard prepared in a small quantity in 1970 (prior to the application of high pressure liquid chromatography to peptide purification) at the University of Chicago [4] was the only well-characterized standard used. We now report comparisons between this old standard and two new human pancreatic standards and a standard of biosynthetic human proinsulin [5].

Materials

The original human proinsulin standard [4] is referred to as standard A in the following. Two new batches of pancreatic human proinsulin were prepared at Novo Research Institute from two side fractions obtained during the preparation of pancreatic human insulin: b-component (standard B; batch No. 30.9. 82) and "citrate I mother liquor" (standard C; batch No. 28.9. 82). Concurrently, biosynthetic human proinsulin [5] was made by recombinant DNA technology at Lilly Research Laboratories (standard D; batch No. 759-OB6-256).

Results and discussion

The proinsulin components of preparations B, C and D were found to elute identically by high pressure liquid chromatography analysis on a Nucleosil C_{18} column with 5 μm particles (Machery-Nagel, Düren, FRG) using an eluent modified from that of Shoelson et al. [6]; the acetonitrile concentration of 32% was approximately 2% higher than that used for the separation of rabbit, human and porcine insulins.

Stock solutions were made of preparations B, C and D and the molar concentrations of proinsulin were determined by quantitative amino-acid analysis. The determinations are considered accurate and precise because (a) they are based on the concentration of 8–10 amino-acids in the hydrolyzed samples relative to the known concentration of the corresponding amino-acids in a standard solution, and (b) because the amino-acid compositions of all three preparations were in agreement with the known composition of human proinsulin.

The N-terminal sequence of amino-acids from residue 1–39 was determined for the proinsulin purified from b-component. The analysis showed only one peptide chain with the amino-acid sequence expected for human proinsulin. The chemical characterization of the biosynthetic human proinsulin has been described previously [5].

Identical standard curves were obtained with preparations B, C and D in the radioimmunoassay of Heding [1] in one laboratory and in the assay of Cohen et al. [3] in two other laboratories. These standard curves were

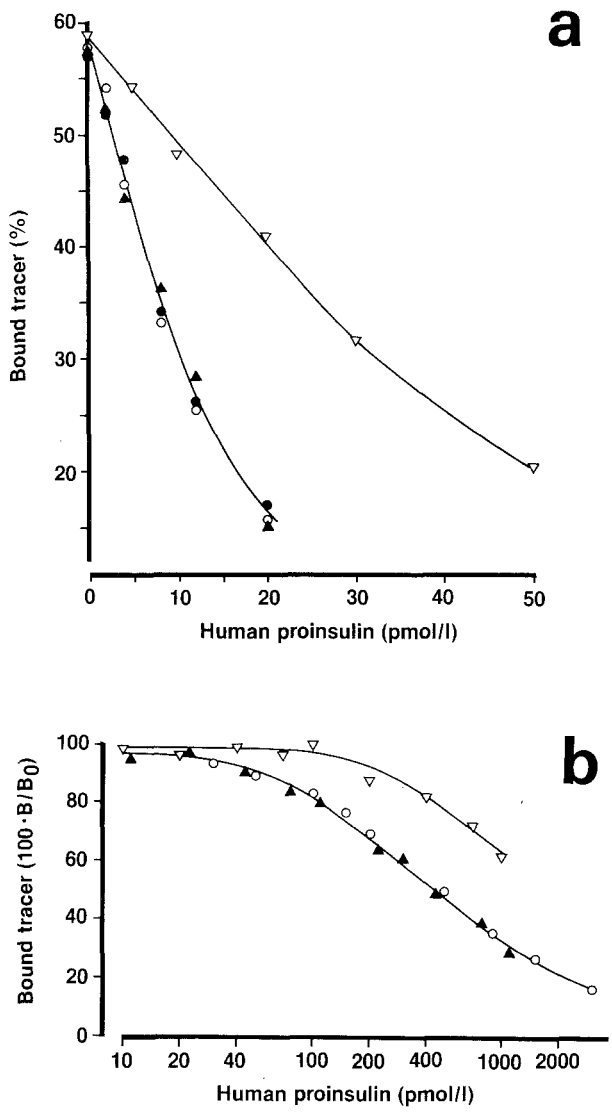

Fig. 1. **a** Standard curves in the proinsulin radioimmunoassay of Heding [1] using antiserum M1219 (linear scale). **b** Standard curves in the proinsulin radioimmunoassay of Cohen et al. [3] using antiserum AO5-OB5-83B and 0.1 ml of sample (logarithmic scale); the binding (B) is expressed as a percentage relative to the binding at zero concentration B_0. Standard A (▽) is the pancreatic human proinsulin as described by Rubenstein and Steiner [4]. Standards B (●) and C (▲) are two new batches of pancreatic human proinsulin, and standard D (○) is biosynthetic human proinsulin [5]

found to differ from those of standard A in the two laboratories in which such comparisons were made (Fig. 1). The differences in the curves indicate that results obtained using the old standard A over-estimate proinsulin concentrations by a factor of three. The factor is likely to be applicable to all data obtained with the assay of Heding [1] because assay quality control indicates that the strength of standard A has been preserved.

These data demonstrate that the new human proinsulin standards may be used interchangeably in the immunoassay of human proinsulin and should supercede previously used standards.

References

1. Heding LG (1977) Specific and direct radioimmunoassay for human proinsulin in serum. Diabetologia 13: 467–474
2. Rainbow SJ, Woodhead JS, Yue DK, Luzio SD, Hales CN (1979) Measurement of human proinsulin by an indirect two-site immunoradiometric assay. Diabetologia 17: 229–234
3. Cohen RM, Blix P, Rue P, Root MA, Frank BH, Revers RR, Rubenstein AH (1983) A specific radioimmunoassay (RIA) for circulating human proinsulin (hPI). Diabetes 32 (Suppl 1): 49 (Abstract)
4. Rubenstein AH, Steiner DF (1970) Human proinsulin: some considerations in the development of a specific immunoassay. In: Camerini-Davalos RA, Cole HS (eds) Early diabetes. Academic Press, New York, pp 159–166
5. Frank BH, Pettee JM, Zimmerman RE, Burck PJ (1981) The production of human proinsulin and its transformation to human insulin and C-peptide. In: Rich DH, Gross E (eds) Peptides: synthesis-structure-function. Pierce Chemicals, Rockford, Illinois, pp 729–738
6. Shoelson S, Haneda M, Blix P, Nanjo A, Sanke T, Inouye K, Steiner D, Rubenstein A, Tager H (1983) Three mutant insulins in man. Nature 302: 540–543

Received: 12 July 1984

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