

Pancreas Transplantation: A Study of Insulin Secretion in Isolated Islets of Langerhans and in Sera Using a New Enzyme-Linked Immunosorbent Assay

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RADIOIMMUNOASSAYS (RIAs) are routinely used for the detection of immunoreactive insulin (IRI).¹ Their disadvantages are limited sensitivity and accuracy and their dependence on instable radionucleotides, requiring a special laboratory. However, for monitoring graft function in pancreas transplantation when immunomodulatory treatment is required a fast, sensitive, constantly available assay is needed. A new enzyme-linked immunosorbent assay (ELISA) method was therefore developed especially for detecting IRI in small volume specimens.

MATERIALS AND METHODS

ELISA Procedure

The ELISA is based on the principle of competitive saturation (Fig 1).

Microtiter plates with 96 round-bottomed wells (Nunc, Denmark) were coated using a sandwich principle. First, the plates were coated with a rabbit anti-guinea pig antibody (antiglobulin antibody) at a pH of 9.6 in a two-hour incubation period at 37°C (150 μ L/well). The plates were washed and then incubated with an anti-insulin antibody (Novo, Denmark) developed in guinea pigs at a final dilution of 1:72,000 and a pH of 7.4, using phosphate-buffered saline (PBS) plus 1% bovine serum albumin (BSA) (100 μ L/well).

Appropriate standards were prepared from the stock solution, for example, starting with 100.0 ng/mL. One hundred microliters of the standard were added in triplicate to the plates after free antibodies had been removed by repeated washings. The samples, rat sera or supernatants from cultivated rat islets, were also diluted in PBS containing 6% BSA and used in triplicate (100 μ L each/well). The plates were allowed to incubate overnight. Next day, 100 μ L of enzyme-labeled insulin (Sigma, St Louis) was added to each well without washing the plates. The incubation lasted for 4 hours at 4°C. Then the plates were washed and 100 μ L orthophenylenediamine dihydrochloride (OPD) solution was added per well. The enzyme reaction was measured at 492 nm.

RESULTS

For a high quality ELISA using an anti-insulin antibody fixed to a solid phase, opti-

mum insulin binding to the anti-insulin antibody is a critical point. It was possible to apply the ELISA principle² to the measurement of insulin by fixing the anti-insulin antibody with an antiglobulin antibody. Curve C in Fig 2 illustrates a typical dose-response curve starting at 100 ng/mL rat insulin. The standard could be diluted to a concentration of 0.049 ng/mL rat insulin, and the curves were clearly dose-dependent. When only buffer was added instead of insulin standard, the optical density (OD) was approximately 0.804. The blanks were no higher than 0.007. This was also true of a standard curve for rat insulin omitting the anti-insulin antibody in the coating procedure. To demonstrate the efficiency of the indirect coating method, curves A and B in Fig 2 show the standard curves that resulted when the anti-insulin antibody was coated directly onto the microtiter plates without using an antiglobulin antibody. These standard curves express only a poor increase in the OD in relation to the amount of insulin.

For routine measuring of supernatants from cultivated rat islets, we used a standard curve ranging from 100 ng/mL to 0.78 ng/mL rat insulin. Using a 1:50 dilution for the culture supernatants, it was possible to detect from 39.0 to 5,000 ng/mL of insulin in one run. For detecting insulin in rat sera, we preferred a standard curve ranging from 12.5 ng/mL to 0.049 ng/mL rat insulin. This means that for sera diluted 1:5, between 0.24 and 60.0 ng/mL of rat insulin could be detected. Further statistical analysis showed that the degree of precision was acceptable,

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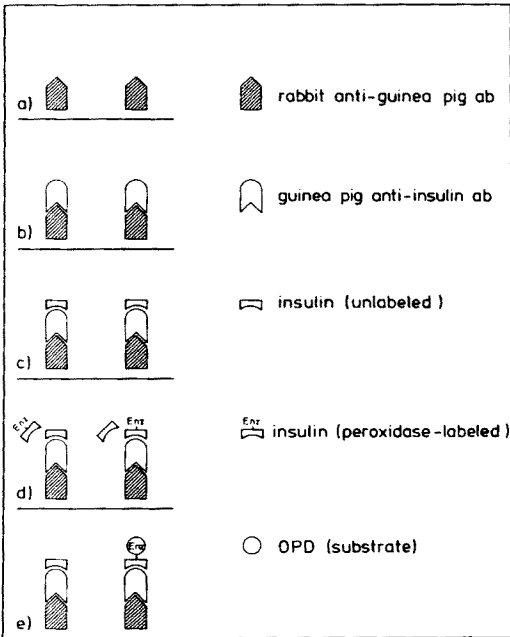


Fig 1. Determination of rat insulin by ELISA: (a) coating of the microtiter plate with the antiglobulin antibody; (b) anti-insulin antibody is bound by the antiglobulin antibody; (c) insulin (from sera, culture supernatants or standard) is bound by the anti-insulin antibody; (d) competitive saturation of the anti-insulin antibody with either unlabeled or enzyme-labeled insulin; and (e) measurement of substrate degradation after removal of all unbound insulin (labeled/unlabeled).

the variation within a test having a standard deviation of $\leq 15.1\%$ and that between different tests of $\leq 15.6\%$. A study on the recovery of rat insulin in serum or culture supernatants was also performed. When the expected values were compared with the ones we measured, the recovery ranged from 80% to 166%.

CONCLUSIONS

Total IRI is conventionally determined by RIA. The known advantages of ELISAs can also be applied to IRI measurement, as demonstrated by a new modified ELISA. It is characterized by indirect binding of the anti-insulin antibody by a second antibody and uses the principle of competitive saturation.

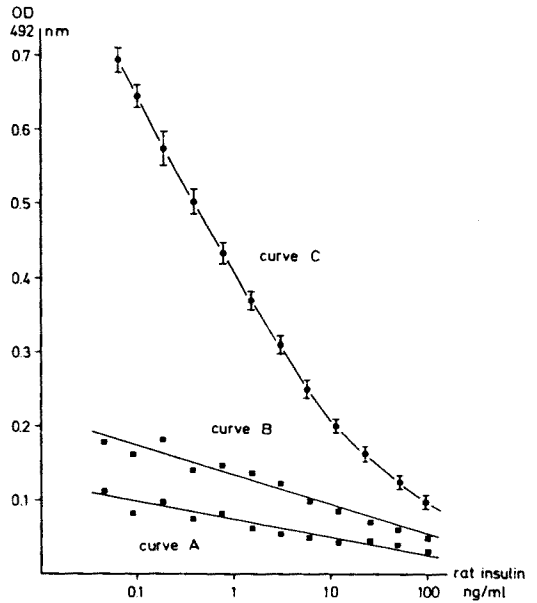


Fig 2. Standard curves: influence of the coating procedure. Curves A and B: the anti-insulin antibody is coated directly onto the microtiter plate without using the antiglobulin antibody (dilution of the anti-insulin antibody for curve B is 1:18,000 and for curve A 1:72,000); Curve C: optimization of the standard curve by fixing the anti-insulin antibody (dilution 1:72,000) with an antiglobulin antibody. OD expressed as mean \pm SD of triplicate samples.

This ELISA is sufficiently accurate, reproducible, and very sensitive. This is the case in detecting both high levels of insulin in culture supernatants and very low levels in rat sera. With the specimens being handled in the microtiter plate system and the lack of dependence on fresh radionucleotides, larger numbers of specimens can be processed at any given time. The new assay thus allows optimized insulin measurement in both in vivo and in vitro experiments, which is essential for planning strategies for pancreas transplantation, for example, immunomodulatory treatment.

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