Rapid Publications

Immunologic Potency of Recombined A- and B-Chains of Synthetic Human and Pancreatic Pork Insulins

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SUMMARY

Pork insulin recombined from cleaved A- and B-chains of pancreatic insulin was shown to be identical with the pancreatic hormone both in erythrocyte receptor assays and in immunoassays employing four different antisera, including two species-specific human anti-insulin sera. Pancreatic human insulin and that prepared from recombined bacterially synthesized A- and B-chains were also indistinguishable in the same systems. This study demonstrates that the prohormone stage of either human or porcine insulin is not required to preserve potency of membrane binding or immunochemical reactivity, even employing an antiserum with marked species specificity. DIABETES *30*:265–266, March 1981.

It had earlier been described that three insulins, pork, dog, and sperm whale, with the same primary amino acid sequences[1] were recognizably different in their cross-reactivities with some antisera but not with others.[2] The possibility of errors in the sequencing of these insulins cannot be ignored. However since the amino acid sequences of the connecting peptides in dog and pig proinsulins are strikingly different,[3] their prohormones are not identical and therefore may manifest conformational differences. It has been suggested[4] that if cleaving of the connecting peptide from the prohormone does not alter the secondary or tertiary structure of the dog or pig insulin, then the configurational differences might be maintained. The present study was undertaken to determine if immunologic differences could be observed between the pancreatic insulins and those derived from recombination of A- and B-chains,[5–7] which are prepared by cleavage of pancreatic insulin or are synthesized by recombinant DNA methodology.

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MATERIALS AND METHODS

Purified pancreatic human (lot #615-1054B-214-1) and pig insulins (lot #614-07J-256), biosynthetic human insulin (lot #615-70N-174-10), and pig insulin (recombined A- and B-chains (lot #615-70N-171-CRY) were obtained from Lilly Research Laboratories through the courtesy of Dr. Mary Root.

The receptor binding potency of each of these preparations was determined using an erythrocyte assay system performed with methods modified slightly from those of Gambhir et al.[8] Monoiodinated (^{125}I) beef insulin[9] was used as tracer and the inhibition of binding of the labeled preparation to erythrocytes by each insulin at concentrations of 2, 5, and 10 ng/ml was determined. All insulin preparations were set up at the same time with the same batch of guinea pig erythrocytes and incubated 12 h at 4°C. Separation of erythrocyte-bound from free ^{125}I-insulin was effected by adding 150 μl of incubation suspension into prechilled microfuge tubes containing 100 μl dibutyl phthalate and 150 μl phosphate-buffered (0.25 M, pH 7.4) normal saline. After centrifugation at 4°C, the tip of each microfuge tube containing the cell pellet was cut off and the radioactivity in the pellet determined.

For the immunologic studies, four antisera that differed in their ability to distinguish among different species of insulins were employed. ^{125}I-beef insulin was used as tracer for all studies and the cross-reaction of the four insulins was determined at the same time with the same dilutions of the insulin stock solutions. The standard diluent employed was 0.02 M barbital buffer (pH 8.6) containing 0.25 mg human serum albumin/ml to prevent nonspecific binding of the labeled insulin to glassware. All assays were incubated for 5 days at 4°C and separation of antibody-bound from free ^{125}I-insulin was effected with charcoal according to methods in general use in the laboratory.

RESULTS

The receptor binding potency, as determined by an erythrocyte assay system of the recombined A- and B-chains of pancreatic insulin, was identical with that of a highly puri-

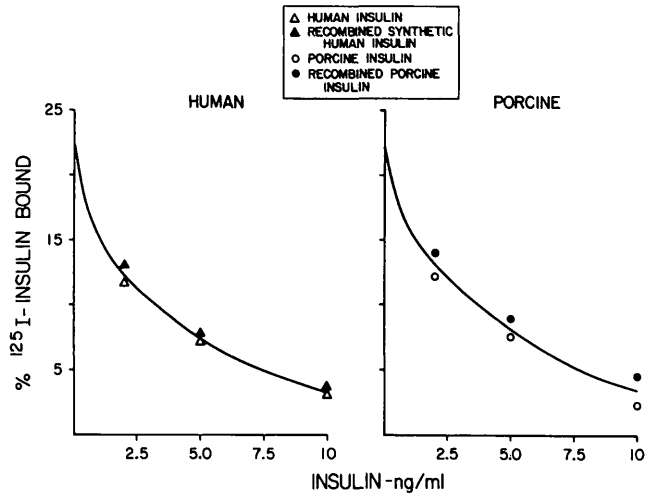
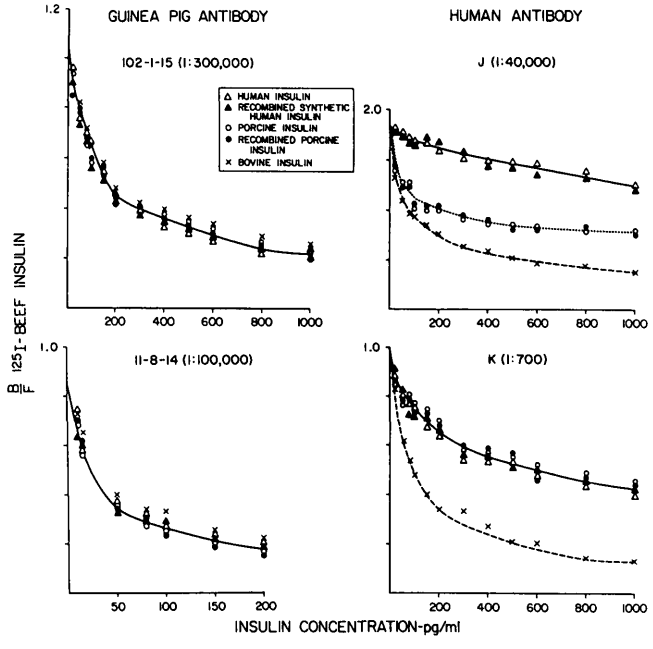


FIGURE 1. Inhibition of binding of ^{125}I-insulin to erythrocyte receptor by pancreatic human insulin and recombined synthetic human insulin (left) and by pancreatic porcine insulin or insulin recombined from the cleaved A- and B-chains of pancreatic porcine insulin (right).

fied preparation of pork insulin (Figure 1, right). When the A- and B-chains of human insulin synthesized by recombinant DNA methodology were combined, the synthetic insulin was indistinguishable from pancreatic human insulin in the same system (Figure 1, left).

For the study of immunologic activity, four antisera were employed: the two guinea pig antisera do not distinguish among bovine, porcine, and human insulins while the human antisera have differing degrees of species specificity (Figure 2). The binding of ^{125}I-beef insulin to human antiserum J is inhibited only slightly by human insulin. At the same insulin concentrations, inhibition is greater with pork insulin and greatest with beef insulin (Figure 2, right top). This antiserum had been shown previously to distinguish readily among pork, sperm whale, and dog insulins.[2] In similar studies, human antiserum K (Figure 2, right bottom) does not distinguish between pork and human insulins, whether pancreatic or synthetic in origin, but shows greater cross-reactivity with beef insulin.

FIGURE 2. Inhibition of binding by several insulins of ^{125}I-beef insulin to guinea pig antisera that do not show species specificity (left) and human antisera with different degrees of species specificity (right).



DISCUSSION

This study demonstrates that the recombined insulins prepared from A- and B-chains, whether derived from pancreatic insulin or from bacterial synthesis, behave identically with intact pancreatic hormone of the same species in the erythrocyte assay system and in several immunologic assay systems. Thus, the directing effect of the connecting peptide may not be necessary to assure the proper tertiary structure for at least some insulins.

The question still unanswered is what accounts for the differences between dog and pork insulins as demonstrable with several human antisera.[2] It could not be due to artifacts introduced by the purification procedures, since plasma and crystalline insulins of the same species were immunochemically indistinguishable. One possibility is that errors have been made in the reported sequences.[1] If this were so, recombining the A-chain of dog insulin with the B-chain of pig insulin and vice versa would permit determination as to which chain has been incorrectly sequenced. Wilson et al.[10] have previously shown that the A-chain may represent a principal site for antigenicity. However, the current study does not rule out the possibility that pork insulin is in the lowest energy state and that recombination of dog A- and B-chains would result in a pork-like insulin.

In summary, this study demonstrates that the prohormone stage of either human or porcine insulin is not required to preserve potency of membrane binding or immunochemical reactivity, even in an assay system employing an antiserum with marked species specificity.

ACKNOWLEDGMENTS

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