Receptor Binding Properties of Human Insulin (recombinant DNA) and Human Proinsulin and Their Interaction at the Receptor Site

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The binding properties of human insulin (recombinant DNA) and human proinsulin were compared in seven healthy volunteers. Specific cell binding of human proinsulin was significantly lower compared with the human insulin receptor binding with an approximately 100-fold lower average affinity (3.71 $mol^{-1} \times 10^8$ for human insulin versus 0.042 $mol^{-1} \times 10^8$ for human proinsulin). Native human proinsulin at low concentrations had no significant effect on specific human insulin receptor binding. Only at high hormone concentrations human proinsulin could displace human insulin from the insulin receptor. We conclude that in vivo proinsulin-insulin interactions at the receptor do not possess major clinical relevance. DIABETES CARE 5 (SUPPL. 2): 104–106, 1982.

Synthesis of human insulin (recombinant DNA) is considered to be a new dimension in insulin therapy of diabetes mellitus. Preliminary data indicate that human insulin might be less immunogenic than porcine insulin in newly diagnosed insulin-dependent diabetic patients.[1] On the other hand, human insulin has shown little differences from purified porcine insulin in terms of biologic activity,[2] resorption from the subcutaneous tissue,[3] and binding to the insulin receptor.[4,5] The possibility of synthesizing human proinsulin by genetic engineering has renewed the interest in possible interactions between insulin and proinsulin at the receptor site and in biologic activity. Although the affinity of proinsulin for the insulin receptor and its biologic activity comprises only about 2% of that of insulin,[6–9] it was recently suggested that human proinsulin at low concentrations may enhance insulin receptor binding.[10] Therefore, the binding properties of biosynthetic proinsulin and a possible interaction with insulin at the receptor site was investigated.

PATIENTS AND METHODS

Insulin and proinsulin binding studies were carried out in seven healthy volunteers (five men, two women). All of them were in their range of normal body weight and none of them took drugs known to effect insulin secretion or carbohydrate metabolism. Blood (120 ml) was drawn after an overnight fast to tubes containing EDTA (dipotassium salt). Mononuclear leucocytes were isolated by gradient centrifugation.[11] Insulin binding studies to mononuclear leucocytes were performed according to the method of Beck-Nielsen et al.[12] The cells were washed twice and incubated in a Hepes buffer (100 mmol/L, pH 7.8, at 15°C) at a concentration of about 5×10^7 cells per milliliter for 100 min. For the binding studies, J125 biosynthetic proinsulin (specific activity: 134 μCi/μg) and J125 TYR A-14 human insulin (specific activity about 227 μCi/μg) as well as native biosynthetic proinsulin and native human insulin were used. Cell-bound and free hormone was separated after the incubation period by centrifugation. The specific cell binding fraction was defined as total cell binding fraction minus nonspecific cell binding, i.e., radioactivity that remained bound in the presence of an excess of native hormone (10 μmol). This fraction averaged about 8% of the total binding for insulin and about 11% for proinsulin. Monocytes were identified by alphanaaphtylesterase staining[13] and specific cell-binding fraction was adjusted to a standard concentration of monocytes of 1.0×10^7/ml using the formula described by Beck-Nielsen et al.[12]

Analysis of the binding data. The results of the human insulin and proinsulin binding studies are presented as specific cell-bound fraction, plotted as a function of total hormone concentration (competition curve). Binding data were further analyzed by the average affinity model of De Meyts.[14]

TABLE 1
Specific cell binding fraction (% of the total radioactivity) and average affinity constant (K_E limiting high-affinity state) of human insulin and human proinsulin

	Specific cell binding fraction ($\times 10^{-2}$)	Average affinity constant K_E ($mol^{-1}/10^8$)
Human insulin	5.42	3.71
Human proinsulin	0.41	0.042

For the comparison of the binding data at different hormone concentrations paired Student's t test was considered appropriate.

RESULTS

The specific cell binding fraction and the average affinity constant of human insulin and human proinsulin are summarized in Table 1. The specific cell binding of human proinsulin comprised only a small proportion of human insulin binding. The average affinity constant of human biosynthetic proinsulin binding was about 100-fold less compared with insulin.

In Figure 1, the effect of increasing amounts of human native biosynthetic proinsulin and native human insulin on insulin receptor binding are compared. In contrast to native insulin, biosynthetic proinsulin showed no significant effect on insulin receptor binding at low hormone concentrations. Only at high concentrations, human proinsulin was able to displace insulin from its receptor.

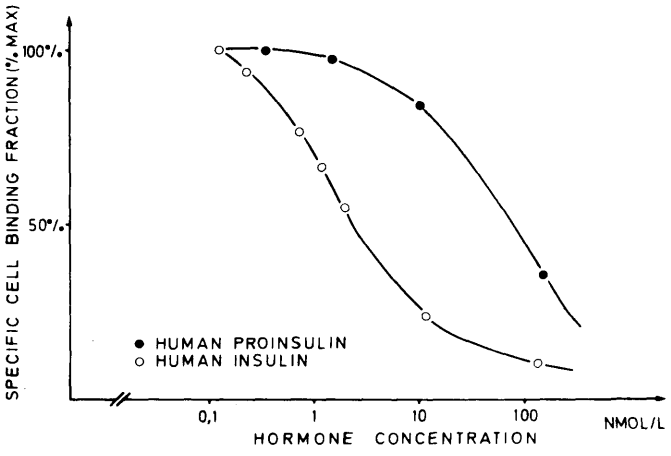

FIG. 1. Effect of increasing amounts of native human proinsulin and native human insulin on specific insulin-receptor binding (^{125}I-human insulin).

DISCUSSION

Proinsulin, the precursor of the insulin molecule, is converted nearly completely to insulin within the B-cell secretory granules, and less than 7% is present in the blood draining the pancreas.[15] A slower metabolism of proinsulin[16] and a lesser hepatic extraction compared with insulin[17] leads to a relative increase in the proportion of proinsulin in the peripheral circulation, where proinsulin can comprise up to 22% of immunoreactive insulin in healthy persons.[18] This more or less constant insulin-proinsulin relationship was reported to be altered in various diseases such as non-insulin-dependent diabetes mellitus[19] or thyrotoxicosis[20] and was believed to be of possible clinical significance in terms of peripheral insulin sensitivity.

In the treatment of insulin-dependent diabetes mellitus, only highly purified porcine and bovine insulin preparations were favored in the last years, since the immunogenicity of most insulin preparations was in part due to specific antibodies against bovine and porcine proinsulin contaminations.[20–24]

The biosynthesis of human insulin as well as human proinsulin offers now the possibility to treat insulin-dependent diabetic patients with a combination of insulin and proinsulin without the fear of immunogenic complications and possibly imitating a more physiologic state. In a preliminary study, Schlüter et al.[10] suggested recently that low proinsulin concentrations may enhance insulin receptor binding to monocytes. This finding was considered to be of clinical significance regarding the biologic activity of human insulin. In contrast to these data, in our study, human biosynthetic proinsulin failed to have any significant influence on insulin receptor binding at low hormone concentrations.

At high, unphysiologic hormone concentrations, proinsulin had an inhibitory effect on insulin receptor binding. The discrepancy with Schlüter et al.[10] cannot be fully explained, but might at least in part be due to methodologic differences.

Using porcine insulin and proinsulin, Beck-Nielsen et al.[12] found a similar competition curve without any effect of low proinsulin concentrations on insulin receptor binding and an inhibitory effect on insulin binding at high hormone concentrations. A low affinity of proinsulin to the insulin receptor with a small biologic activity was also reported in studies of Gliemann and Gammeltoft, who also found no evidence for a stimulatory effect of low proinsulin concentrations on insulin-receptor binding to isolated hepatocytes.[8,9]

From the clinical point of view, we conclude from our data that the combined administration of human insulin and human proinsulin in the treatment of insulin-dependent diabetes mellitus does not possess major clinical relevance regarding peripheral insulin binding and, furthermore, biologic activity.

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