Guinea Pig Insulin

II. BIOLOGICAL ACTIVITY*

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SUMMARY

The biological activity of guinea pig insulin was determined to be between 1 and 3 i.u. per mg when measured in vitro using isolated rat tissues and in vivo by mouse convulsion. However, when assayed by blood glucose lowering in the guinea pig, guinea pig insulin has a potency of 8.5 to 9.0 i.u. per mg. The low activity in vitro was not due to a more rapid degradation of guinea pig insulin by the rat tissues. The dose-response relationship in the isolated epididymal fat cell assay showed that guinea pig insulin had an intrinsic biological activity comparable to that of bovine insulin, but a reduced affinity for the tissue receptor sites.

We have shown that the physical properties of guinea pig insulin are uniquely different from those of bovine or porcine insulin as determined by zinc binding studies, ultraviolet difference absorption spectrophotometry, and ultracentrifugation analysis (1, 2). It is likely that such differences in physical properties are accompanied by changes in biological activity. Consequently we decided to study the biological activity of our preparation of guinea pig insulin. The potency of guinea pig insulin has been reported to be lower than that of bovine insulin (3, 4). However, the purity of the preparation used was not known and the standard errors or confidence limits of the bioassays were not reported.

In the present study, we re-examined the biological activity of homogenized guinea pig insulin in four bioassays in vitro using rat and guinea pig tissues and in two bioassays in vivo using the mouse and guinea pig. A dose-response study was carried out to determine if the low biological activity of guinea pig insulin was due to a low intrinsic activity or to an altered affinity for the hormone receptors.

MATERIALS AND METHODS

Guinea pig insulin used was obtained as described (2). Crystalline bovine insulin standard, lot No. 79, was obtained as a gift from Connaught Medical Research Laboratories, Toronto, Ontario. Carrier-free 125I was purchased from New England Nuclear Corp.

Bioassays of Insulin in Vitro

Rat Hemidiaphragm Assay—The biological activity of the purified guinea pig insulin was assayed by the isolated rat hemidiaphragm method described by Shaw and Chance (5) using a six-point design with quadruplicate samples. The potency of the guinea pig insulin preparation was calculated by the method described by Bliss and Marks (6, 7) and a complete analysis of variability was performed (8, 9).

Rat Epididymal Fat Pad Assay—This assay was performed as a four-point assay in quadruplicate according to the method of Renold et al. (10).

Epididymal Fat Cell Assay—Isolated fat cells were prepared from rats weighing 150 g or from guinea pigs weighing 500 g according to Gilman's (11) modification of the method of Rodbell (12). For assay, approximately 106 adipocytes were incubated for 90 min at 37° under 95% O2-5% CO2 in 2 ml of modified Krebs-Ringer bicarbonate buffer containing one-half the usual concentration of calcium, 1 mg per ml of dialyzed crystalline human serum albumin (Nutritional Biochemical Corp.), 0.1 mg per ml of glucose, and 0.4 μCi of n-[U-14C]glucose (New England Nuclear). This assay was performed as a six-point assay in triplicate.

Bioassay of Insulin in Vivo

Mouse Convulsion Assay—The relative biological potency of guinea pig insulin was determined using the mouse convulsion technique described by Young and Lewis (13). The assays were performed by the Connaught Medical Research Laboratories, Toronto, through the kind cooperation of Dr. A. M. Fisher.

Guinea Pig Blood Glucose Lowering Assay—Since it is difficult to obtain repeated blood samples from the guinea pig without excessive stress upon the animal, the method of Young and Romans (14) requiring only one blood sample from each of 12 animals on each of 4 test days was adopted. The experimental design was patterned after that described by Bliss and Marks (6, 7). The potency of guinea pig insulin was calculated on the assumption that the bovine insulin standard possessed an activity of 22.83 i.u. per mg in the guinea pig, as it did in the mouse convulsion assay.

Degradation of 125I-Insulin by Rat Adipose Tissue in Vitro

Experiments were carried out to measure the rates of disappearance of 125I-labeled bovine and guinea pig insulin from the medium when incubated with rat adipose tissue to determine if differences in the rates of degradation of these insulins might account for differences in biological potency. Fragments of rat epididymal fat pad were prepared as for the fat pad assay and were first incubated for 15 min in Krebs-Ringer bicarbonate buffer under 95% O2-5% CO2 at 37°. Radiiodinated bovine or guinea pig insulin, prepared and purified by the method of Hunter and Greenwood (15), was added to each sample vial, at a concentration of 22.5
Results

Estimate of Biological Potency—The potency estimates for guinea pig insulin assayed in vitro against bovine insulin standard in three systems using rat tissues and one using guinea pig tissue were about 1 to 2 i.u. per mg (Table 1). This is in good agreement with the results of bioassays in vivo by mouse convolution, where the potency was 2.12 i.u. per mg. Based on a potency of 22.83 i.u. per mg for the bovine insulin standard, guinea pig insulin therefore is about one-tenth as potent in these assays. However, when guinea pig insulin was tested in a “cross-over assay” against bovine insulin for its ability to lower the blood glucose concentration in the guinea pig, the potency was 8.58 i.u. per mg.

The low biological activity of guinea pig insulin on rat adipose tissue in vitro could not be attributed to its more rapid degradation by the tissue. As shown in Fig. 1, the degradation and disappearance of 125I-labeled guinea pig insulin or bovine insulin were similar, with less than 10% of the labeled insulin having disappeared from the medium during the first 30 min, through either degradation or uptake by the tissue. In fact, the rate of disappearance and degradation of guinea pig insulin appeared to be slower than that of bovine insulin.

Neutralization of Biological Activity by Specific Antiserum—Guinea pig anti-bovine insulin serum markedly inhibited the response of the tissue to bovine insulin (p < 0.05) but did not affect the response to guinea pig insulin (Fig. 2). The anti-guinea pig insulin serum produced in the rabbit inhibited only the response of the tissue to guinea pig insulin (p < 0.01) and did not neutralize the biological activity of bovine insulin. Normal rabbit serum did not alter the response of the tissue to

Table I

<table>
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<th>Bioassay</th>
<th>Potency</th>
<th>95% confidence limit</th>
<th>Index of precision (λ)</th>
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<td>Rat hemidiaphragm</td>
<td>1.35</td>
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<td>6.06-12.16</td>
<td>0.20</td>
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<td>Mouse convulsion</td>
<td>2.12</td>
<td>1.43-3.18</td>
<td></td>
</tr>
</tbody>
</table>

* Based on the assumption that bovine insulin standard possessed an activity of 22.83 i.u. per mg in the guinea pig.

Fig. 1. The degradation and disappearance of 125I-labeled bovine insulin (●) and 125I-labeled guinea pig insulin (▲) when incubated with rat epididymal adipose tissue. Degradation was measured as the increase of radioactivity soluble in trichloroacetic acid. Disappearance was measured as the percentage of radioactive immunoreactive material lost from the incubation medium. The results are expressed, respectively, as percentage of the radioactive material insoluble in trichloroacetic acid and immunoprecipitable by the appropriate antiserum at zero time in the absence of tissue.

Fig. 2. Test of specificity of rabbit anti-guinea pig insulin serum, guinea pig anti-bovine insulin serum, and normal rabbit serum in neutralizing the biological activity of guinea pig insulin and bovine insulin in the rat epididymal fat pad assay. Results are expressed as the mean ± S.E., triplicate determinations, of 14CO2 production in disintegrations per min per mg of tissue wet weight. The bar labeled C represents the 14CO2 production in control incubated without insulin. The inhibition by the specific antiserum was significant at the 5% level and 1% level, respectively, in the case of bovine insulin and guinea pig insulin.
either guinea pig or bovine insulin. Comparable results were obtained in a similar test of the same antisera in the rat hemidiaphragm assay.

**DISCUSSION**

The present study shows that the biological activity of guinea pig insulin is considerably lower than that of bovine insulin in several standard bioassays and that guinea pig insulin is immunologically distinctive. The biological activity of guinea pig insulin is neutralized by rabbit anti-guinea pig insulin serum, but not by guinea pig anti-bovine insulin serum. Rabbit anti-guinea pig insulin serum does not cross-react with bovine insulin. These results confirm earlier studies *in vitro* (16–21) and *in vivo* (22, 23) that guinea pig insulin was not neutralized by anti-bovine or other anti-insulin sera produced in the guinea pig.

Insulins from other mammals studied so far are known to possess biological activities comparable to that of bovine insulin. Furthermore, the insulins of many nonmammalian species exhibit biological activities that are close to that of the mammalian insulins, although several of these insulins, such as insulin from the chicken (16, 24), bonito fish (25), cod (16, 26, 27), pollock (16, 26), and anglerfish (28), differ considerably in amino acid sequence and in immunological properties from the mammalian insulins. However, some nonmammalian insulins may have low biological activity. Hagfish insulin has been shown to have a potency of about 2 i.u. per mg (20) and the insulins of the frog and dogfish may also have low biological activity (16).

It is interesting that guinea pig insulin is not as potent as bovine insulin in the guinea pig itself. The effect of guinea pig insulin on glucose oxidation by isolated guinea pig epididymal fat cells was less than 5% that of bovine insulin, while its hypoglycemic effect in the intact guinea pig was almost 40% that of bovine insulin. This observation is in sharp contrast to the reports that bovine insulin was less active than chicken insulin in the chicken (24) and less active than hagfish insulin in the hagfish (16, 30).

It was reported that the potency of cod insulin was about the same whether assayed by guinea pig blood glucose lowering, by the rat epididymal fat pad, or by the mouse convulsion technique, when compared with a bovine insulin standard (31). It would appear, therefore, that despite great differences in immunological and physical properties between guinea pig insulin and the other mammalian and certain nonmammalian insulins, the guinea pig is more sensitive to the hypoglycemic effect of these insulins than of its own insulin. This suggests that the insulin receptor sites of the guinea pig tissues may recognize insulins of other species more efficiently than its own insulin. This in turn implies that the insulin receptors of the guinea pig might not have evolved in parallel with the insulin of this animal. However, insulin receptors in the guinea pig may be different from those of other species, such as the mouse. This can be inferred from observations that guinea pig insulin is more potent in lowering the blood glucose of the guinea pig than of the mouse and that insulin treated with 5-dimethylaminonaphthalene-1-sulfonyl at the B-1 phenylalanine position retained only 48% of its activity in the mouse convulsion assay but was fully active in the guinea pig (32).

The amino acid residues at the hypothetical active site of insulin (33) are conserved in guinea pig insulin, except for the D-22 arginine which is replaced by aspartic acid. In crystalline porcine insulin hexamers the guanidinium of this arginine interacts with the carboxylate group of the A-21 asparagine. Removal of the A-21 asparagine residue greatly disrupts the structure of the molecule (34, 35), impairing its ability to dimerize, and results in nearly total loss of biological activity (36). Therefore, the replacement of the B-22 arginine by aspartic acid in guinea pig insulin may be expected to cause a loss in biological activity as well and could contribute to its inability to form dimers (1, 2). It is not known if the low biological activity of guinea pig insulin may be a consequence of its failure to dimerize, perhaps at the receptor site, although monomeric insulin is likely the active species (37, 38). The low biological activity of guinea pig insulin may be the result of more complex structural alterations which have not yet been elucidated.

The biological activity of a hormone is determined both by its intrinsic biological activity and by its binding affinity to its tissue receptors (39, 40). Either a reduced intrinsic activity or reduced binding affinity may account for the low potency of guinea pig insulin as compared to that of bovine insulin. The dose-response relationships determined in the bioassays suggest that guinea pig insulin has the same intrinsic activity as bovine insulin but possesses a lower binding affinity. Guinea pig insulin...
has been shown to compete less effectively than bovine insulin for binding to rat liver plasma membrane (41, 42). Thus, the active site of guinea pig insulin may be similar to that of bovine insulin, whereas the binding site may be different. It is not known, however, if the active site and the binding site are discrete areas of the insulin molecule, as is the case with adrenocorticotropic hormone (43) and glucagon (44).

In conclusion, the insulin of the guinea pig is considerably less active biologically than bovine insulin. The physiological significance of this observation in the guinea pig remains unknown. However, it is of interest to note that the insulin concentration in sera from a population of guinea pigs ranges between 21 and 136 ng per ml, with a mean ± S.D. of 52 ± 37 ng per ml, as measured by radioimmunoassay using the rabbit anti-guinea pig insulin serum (45). This serum insulin concentration is more than 10-fold higher than that in other animals examined (46). The high level of circulating insulin in the guinea pig may compensate for the low biological activity of the hormone.

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REFERENCES
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