Insulin-like growth factor I and II in 14 animal species and man as determined by three radioligand assays and two bioassays

Ines Zangger, Jürgen Zapf and E. Rudolf Froesch

Metabolic Unit, Department of Medicine, University Hospital, University of Zürich, Zürich, Switzerland

Abstract. Insulin-like growth factor I and II (IGF I and II) were determined by five different assays in human serum, in the sera of ten mammalian species and in chicken, turtle, and frog serum. Sera of all tested mammals contain two different IGFs corresponding to human immunoreactive IGF I and receptor reactive IGF II. Receptor reactive IGF II of most animal species does not show significant cross-reactivity in the RIA for human IGF II. IGF activity was also detected in sera of non-mammals, such as chicken and turtles, but not in frog serum. The IGF values obtained with the different assay system corresponded rather well: there is a good correlation between the values obtained in the protein binding and the fat cell assay, and between the results of the latter assays and the sum of immunoreactive IGF I and receptor reactive IGF II. The results suggest that those regions in the IGF I and II molecules which are responsible for reactivity with the type I IGF and the insulin receptor have not essentially changed during evolution. Similarly, the C-region, which mainly determines the immunological properties of IGFs, appears to have remained relatively constant in the IGF I, but not in the IGF II molecule.

Two major insulin-like growth factors, IGF I and II, have been characterized in human serum (Rinderknecht & Humbel 1978a,b). Genetic heterogeneity has been shown in the case of IGF II (Zumstein et al. 1985; Jansen et al. 1985). IGF I is identical with somatomedin C (Klapper et al. 1983) and somatomedin A (Enberg et al. 1984). It is present in normal serum in a concentration of ~200 µg/l (Zapf et al. 1980, 1981). IGF I levels are increased in acromegaly and decreased in hypopituitarism (Zapf et al. 1980, 1981). IGF is synthesized and rapidly secreted by the perfused rat liver, and synthesis and secretion are dependent on the growth hormone status (Schwander et al. 1983). IGF I is more dependent on growth hormone than IGF II. It is at least three times more potent in vitro and in vivo than IGF II in stimulating growth indices in rat cartilage and rat bone (Zapf et al. 1978; Schmid et al. 1983; Schoenle et al. 1985).

IGF I and II are close relatives of insulin. They contain A- and B-regions which display 43 and 41% homology with the A- and B-chain of insulin. In contrast to insulin, IGF I and II contain a C-region consisting of 12 and 8 amino acids, respectively. Both molecules have an additional prolongation at the C-terminus of the A-chain, termed D-region (Rinderknecht & Humbel 1978a,b).

The goal of this study was to measure IGF I and IGF II serum levels in different animal species by the available bio- and radioligand assays which recognize different parts of the IGF molecules. Comparative determinations of IGF I and II have been carried out by Wilson & Hintz (1982) with radioimmunoassays (RIA) using antibodies against the C-regions of human IGF I and II.

Materials and Methods
Sera
Table 1 shows the list of sera in which IGF I and IGF II were determined. The sera were obtained from the Veterinary Hospital and from the Department of
Table 1.  
Total IGF (PBA, FCA, CEFA), IGF I (RIA I) and IGF II (RIA II, RRA) in serum extracts of 14 animal species and man.

<table>
<thead>
<tr>
<th>Species</th>
<th>PBA (mU/l)</th>
<th>FCA (mU/l)</th>
<th>RIA I (μg/l)</th>
<th>IRA II (μg/l)</th>
<th>RRA (μg/l)</th>
<th>CEFA (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>892</td>
<td>622</td>
<td>459</td>
<td>573</td>
<td>920</td>
<td>543</td>
</tr>
<tr>
<td>Great dane</td>
<td>652</td>
<td>414</td>
<td>332</td>
<td>27</td>
<td>747</td>
<td>936</td>
</tr>
<tr>
<td>Labrador</td>
<td>485</td>
<td>363</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>396</td>
</tr>
<tr>
<td>Terrier</td>
<td>445</td>
<td>276</td>
<td>184</td>
<td>n. d.</td>
<td>n. d.</td>
<td>153</td>
</tr>
<tr>
<td>German shepherd</td>
<td>407</td>
<td>239</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>435</td>
</tr>
<tr>
<td>'dog'</td>
<td>368</td>
<td>261</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>492</td>
</tr>
<tr>
<td>Man</td>
<td>313</td>
<td>267</td>
<td>177</td>
<td>647</td>
<td>685</td>
<td>243</td>
</tr>
<tr>
<td>Sheep</td>
<td>278</td>
<td>244</td>
<td>119</td>
<td>n. m.</td>
<td>201</td>
<td>168</td>
</tr>
<tr>
<td>Pony</td>
<td>242</td>
<td>201</td>
<td>124</td>
<td>&lt; 10</td>
<td>279</td>
<td>78</td>
</tr>
<tr>
<td>Monkey</td>
<td>202</td>
<td>183</td>
<td>127</td>
<td>389</td>
<td>404</td>
<td>45</td>
</tr>
<tr>
<td>Cow</td>
<td>126</td>
<td>154</td>
<td>20</td>
<td>10</td>
<td>278</td>
<td>69</td>
</tr>
<tr>
<td>Horse</td>
<td>121</td>
<td>66</td>
<td>115</td>
<td>&lt; 10</td>
<td>149</td>
<td>72</td>
</tr>
<tr>
<td>Cat</td>
<td>111</td>
<td>99</td>
<td>73</td>
<td>n. m.</td>
<td>126</td>
<td>t</td>
</tr>
<tr>
<td>Rabbit</td>
<td>97</td>
<td>65</td>
<td>10</td>
<td>n. m.</td>
<td>47</td>
<td>15</td>
</tr>
<tr>
<td>Rat</td>
<td>89</td>
<td>30</td>
<td>10*</td>
<td>&lt; 10</td>
<td>n. d.</td>
<td>207</td>
</tr>
<tr>
<td>Goat</td>
<td>91</td>
<td>88</td>
<td>22</td>
<td>9</td>
<td>121</td>
<td>15</td>
</tr>
<tr>
<td>Chicken</td>
<td>24</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>t</td>
</tr>
<tr>
<td>Turtle</td>
<td>15</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>t</td>
</tr>
<tr>
<td>Frog</td>
<td>n. m.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>t</td>
</tr>
</tbody>
</table>

n. d.: not determined; n. m.: not measurable; t: toxic (values below baseline).

* When measured with the antiserum of Reber & Liske (1976) IGF I levels in normal rats lie between 130–200 µg/l.

Physiology of the University of Zürich. Blood was drawn and kept at 4°C for 1 h before it was centrifuged for 15 min at 2000 × g. The serum was stored frozen at −20°C.

All sera (0.5−1.0 ml) were chromatographed on Sephadex G-50 medium columns (1.5 × 100 cm, flow rate 20 ml/h) in 1 mol/l acetic acid at 4°C. Fractions of 2 ml were collected. Fractions eluting between 50−55, 55−60 and 60−80% bed volume were pooled, lyophilized, dissolved in 2 ml of 0.1 mol/l NH₄HCO₃, and relyophilized. For the protein-binding assay each of the three pools was dissolved in 1 ml of 0.1 mol/l NH₄HCO₃ and assayed at three different dilutions. Equal aliquots of the three pools were then combined, relyophilized and again dissolved in 1 ml of 0.1 mol/l NH₄HCO₃ for the other assays.

Assays

1) **Protein-binding assay (PBA).** The protein-binding assay was performed with a partially purified preparation of human IGF binding protein as described earlier (Zapf et al. 1977). [125]IGF I was used as a tracer and a partially purified IGF preparation (specific biological activity in the rat fat pad assay 4.5 mU/mg) containing a 1:1 mixture of IGF I and II (as determined by RIA) was used for standing dilutions. IGF values were therefore read from the standard curve as µU equivalents of insulin-like activity. For human serum, values obtained in the PBA are approximately twice as high as values obtained in the fat pad assay (Zapf et al. 1977, 1980) owing to potency differences between IGF I and II in the PBA, but not in the fat pad assay (Zapf et al. 1978). Since 1 µU equivalent in the fat pad assay corresponds to ~ 3 ng of IGF I or II (Zapf et al. 1978), µU equivalents obtained in the PBA can be converted to ng equivalents of total IGF by multiplication with a factor of 1.5. The same holds true for µU equivalents determined in the fat cell assay (see below).

2) **Fat cell assay (FCA).** IGF was determined in the fat cell assay in the presence of insulin antiserum (Zapf et
al. 1980). Fat cells were obtained from epididymal fat pads of 120–130 g male Zdr Cara rats by collagenase digestion. Whale insulin (same amino acid sequence as porcine insulin) was used as a standard. Similar to the PBA, µU equivalents obtained for human serum in the FCA are twice as high as the results of the fat pad assay (Zapf et al. 1980). Again, this discrepancy is due to the potency difference between IGF I and II in the FCA (Zapf et al. 1978).

3) Radioimmunoassay (RIA). The radioimmunoassays for IGF I and IGF II were carried out as described by Zapf et al. (1981). Pure IGF I and II (generously provided by Dr. R. Humbel, Zürich) served as standards.

4) Radioreceptor assay (RRA). Rat liver membranes were used for the determination of receptor-reactive IGF II as described by Widmer et al. (1982). [125I]IGF II was used as a tracer and pure IGF II as a standard.

5) Chick embryo fibroblast assay (CEFA). [3H]thymidine incorporation into stationary primary chick embryo fibroblasts was determined according to the method of Morell & Froesch (1973). The assay was standardized with pure IGF I.

Of most sera several aliquots were chromatographed in order to obtain enough material for the assays. The material was tested at 2 or 3 different dilutions in most of the assays. Total IGF was determined 4 times in the PBA and the mean of these determinations is given. IGF I was measured in two independent RIA.

Results

All results are shown in Table 1. Total IGF values obtained in the PBA and in the FCA showed a highly significant correlation (r = 0.975, P < 0.001) (Fig. 1). In the sheep, cow, cat, goat, monkey, and in man the values determined in the PBA were comparable to those of the FCA and the ratio was close to 1.0 (0.83–1.17). In the guinea pig, in the different species of dogs, in the horse, and in the rabbit the ratio between the values of the PBA and the FCA varied from 1.4 to 1.8. In the rat, the ratio was 3. Obviously, the relative insulin-like biological activities mediated by the insulin receptor of the fat cell are smaller for IGFs of the latter species. When compared with the biological activities in the CEFA, which are mediated by the type 1 IGF receptor, rat IGF appears to be the most potent IGF.

Most animals, except monkeys and guinea pigs, had no measurable IGF II in the RIA for human IGF II. Therefore, IGF II was measured by RRA. A highly significant correlation was found between the results obtained for total IGF in the PBA or the FCA and the sum of the results of the RIA for IGF I and the RIA for IGF II (Fig. 2, r = 0.946, P < 0.001; not shown for the FCA). µU
equivalents of IGF as determined in the PBA or FCA were converted into ng equivalents using the ratio for human IGF (see Materials and Methods). The ratios between total IGF in the PBA and the sum of IGF I (RIA) plus IGF II (RRA) are between 0.55–0.69 in man, monkey, cow, and horse, and 0.84–1.3 in the cat, goat, dog, guinea pig, and sheep.

All tested serum extracts with the exception of those of cat, turtle and frog stimulated [H]thymidine incorporation into DNA of chick embryo fibroblasts. Those of the cat, turtle, and frog were inhibitory and thus apparently toxic.

Discussion

IGFs can be separated from their binding proteins in all animal species studied if the sera are gel-filtered on Sephadex G-50 at acidic pH.

All mammals have measurable IGF levels in the PBA and in the FCA, and the correlation between the values obtained in these two assays is highly significant. The results of the PBA or FCA which measure both IGF I and IGF II can be compared with the sum of the results of the RIA I plus those of the RRA II. As expected, the correlation is highly significant (Fig. 2; not shown for the FCA).

These data support the contention that the biologically active site (interacting with the insulin or type I IGF receptor) and the site responsible for the interaction with the binding protein are related within mammalian IGF molecules and parts of the same molecular region (Froesch et al. 1979).

There are IGF molecules in the serum of all tested mammals that react in the RIA for human IGF I. Since IGF antibodies recognize mostly the C-region (Honegger & Humbel 1986), this area in the IGF I molecule must have been well conserved during evolution.

Another interesting aspect is that only guinea pig and monkey sera contain material that cross-reacts in the RIA for human IGF II. Apparently, the C-region in the IGF II molecule has undergone considerable evolutionary alterations. Following up this line of reasoning, it is not astonishing that monkey IGF II cross-reacts with antihuman IGF II antibodies. However, the cross-
reactivity of guinea pig IGF II is unexpected. On the other hand, there appear to be IGF II-like molecules in all tested mammals that cross-react in the RRA for IGF II. In man, three slightly different IGF II molecules have been detected, probably resulting from different ways of IGF II mRNA splicing as well as from two allelic IGF II genes (Jansen et al. 1985; Zumstein et al. 1985). These human IGF II species appear to be active in the PBA, FCA, RIA II and RRA II. So far, we do not know which equivalent of the IGF II species of human serum is the abundant form in the serum of mammals.

The amino acid sequences in the A- and B-region that are important for the binding to the receptor and for the biological activity appear to have been maintained relatively constant in both IGF I and IGF II (Rinderknecht & Humbel 1978b; Blundell et al. 1983). It appears likely that mutations in these regions that lead to a loss of the biological potency of IGF are deleted by nature.

Rat serum extracts exhibit some peculiarities. They are two to three times less active in the PBA than in the FCA. Also, they do not yield parallel displacement curves in the RIA I. On the other hand, rat IGF is very active on chick embryo fibroblasts and three times more active than human IGF I on human fibroblasts and also on rat calvaria cells (Schmid et al. 1983). Rat IGF appears to differ from human IGF more than guinea pig IGF in all assay systems. This is particularly interesting in view of guinea pig insulin which is very remote from human insulin: their amino acid sequences differ in 14 out of 54 positions. In this context, it is surprising that guinea pig serum extracts cross-react in the IGF II RIA, indicating a considerable degree of homology between the human and the guinea pig IGF II molecules in the C-region. Another interesting point concerns the high levels of IGF I and IGF II in guinea pig serum. One might speculate that the guinea pig IGFs have retained some of the physiological functions that have been taken on by insulin in other mammals.

DNA-synthesis of stationary chick embryo fibroblasts is stimulated by acid chromatographed sera of all tested species, with the exception of the cat, turtle and frog. However, these results must be viewed with caution: The serum extracts of all species, particularly those of the cat, turtle and frog were toxic at high concentrations, i.e. they inhibited 3H-thymidine incorporation into DNA. It is, therefore, likely that IGF levels determined in the CEFA are underestimated. It must also be kept in mind that total IGF was measured, most of which is bound to a 150–200 kDa binding protein in human and rat serum (Zapf et al. 1984; Moses et al. 1979). Other species may have other or no binding proteins and correspondingly lower total IGF serum levels.

The last point of interest concerns the fact that the amount of IGF extracted from serum of several species does not correlate with the size of the animals. Thus, a small animal such as the guinea pig has the highest levels of IGF I and IGF II, whereas big animals such as beef and horse have relatively low levels. Nevertheless, it appears that IGF I has something to do with body size when it is compared within the same race. Eigenmann et al. (1984b) found that the standard poodle has higher levels of IGF I than the miniature poodle and the latter has higher levels than the toy poodle. IGF I levels correlate also with the size of different dog breeds (Eigenmann 1984a). These data on the different sized dogs and poodles are in agreement with the low IGF I levels in the pigmies and in white people with the pigmy trait (Merimee et al. 1982). Thus, within a given species, the IGF I levels may well determine the size of individual animals provided a comparable environment and good nutrition.

Acknowledgments

This work was supported by Grant No. 3.051-0.84 of the Swiss National Science Foundation.

We wish to thank Ms R. Meister and M. Salman for secretarial help, Ms Ch. Hauri, Th. Steiner, and Dr U. Widmer for methodologic assistance, and Dr E. Isenbügel and Dr H.-R. Lüscher for their help in collecting sera from various animals.

References


Received April 17th, 1986. Accepted August 21st, 1986.

Dr E. R. Froesch, Metabolic Unit, Department of Medicine, University Hospital, University of Zürich, CH-8091 Zürich, Switzerland.