Plasma clearance and tissue distribution of labelled chicken and human IGF-I and IGF-II in the chicken

J P McMurtry, G L Francis, Z Upton, P E Walton, G Rosselot, T J Caperna and D M Brocket

United States Department of Agriculture, Growth Biology Laboratory, Agricultural Research Service, Beltsville, Maryland 20705, USA, *Cooperative Research Centre for Tissue Growth and Repair and CSIRO, Division of Human Nutrition, Adelaide, South Australia 5000, Australia and Instituto de Nutricion y Tecnologia de los Alimentos, Universidad de Chile, Macul, Santiago, Chile

(Requests for offprints should be addressed to J P McMurtry, US Department of Agriculture, Growth Biology Laboratory, BARC-East, Building 200, Beltsville, Maryland 20705, USA)

Abstract

The metabolic clearance of chicken IGF-I (cIGF-I), cIGF-II, human IGF-I (hIGF-I), and hIGF-II was examined in the chicken using 125I-labelled growth factors. Superose-12 chromatography of plasma collected at 7.5 min post-infusion revealed peaks of radioactivity corresponding to 150 and 43 kDa and unbound tracer. Statistical analysis of trichloroacetic acid (TCA)-precipitable radioactivity in sequential plasma samples as well as following chromatography of the same samples revealed that clearance of the radiolabelled peptides followed an apparent triphasic pattern. The close similarity of the individual chromatographically defined pools in their clearance rate compared with the three components described by TCA precipitation strongly suggested their identity. Both free 125I-labelled cIGF-II (3-11 min) and hIGF-II (3-01 min) were cleared at a greater rate than their IGF-I counterparts. Unbound hIGF-I was cleared at a greater rate than cIGF-I (4.45 vs 5.66 min respectively). A similar pattern for clearance was evident in the radiolabelled growth factors associated with the 43 kDa component, although at a longer half-life. There was no difference in the apparent clearance of the radiolabelled growth factors associated with the 150 kDa component between IGF-I or -II or between species. Analysis of the chromatographic profiles of radioactive IGF-I peptides complexed to serum proteins versus those bound to labelled IGF-II peptides revealed the presence of a large molecular mass binding protein in vivo. Ligand blotting of chicken serum determined that a binding protein with a mass of 70 kDa was detectable with 125I-IGF-II probes only, and was not present in pig serum. In addition, tissue uptake of 125I-cIGF-I and -II was evaluated. Similar patterns of tissue distribution and uptake were observed for 125I-cIGF-I and -II, except that cIGF-II uptake by the liver exceeded that of 125I-cIGF-I at 15 min post-infusion. The rank order of tissue distribution was as follows: kidney→testis→heart→liver→pancreas→small intestine→cartilage→bursa→gizzard→leg muscle→breast muscle→brain. We conclude from these studies that the clearance of IGFs from the compartments identified in blood and the potential target tissues is dependent on their interactions with IGF-binding proteins and receptors.

Journal of Endocrinology (1996) 150, 149–160

Introduction

The insulin-like growth factors (IGF-I and -II) are polypeptides whose primary, secondary and tertiary structures are strikingly homologous to proinsulin. IGF-I is a 70 amino acid peptide which plays a significant role in mammalian growth as a mediator of growth hormone, either as an endocrine or autocrine-paracrine effector (Daughaday & Rotwein 1989). The structure of mature chicken IGF-I (cIGF-I) was reported to be comprised of 70 amino acids, with eight amino acids differing from human, porcine or bovine IGF-I (Ballard et al. 1990). The amino acid sequence of the mature protein confirmed that deduced from cIGF-I cDNAs (Kajimoto & Rotwein 1989, Fawcett & Bulfield 1990). IGF-II, which contains 67 amino acids, is structurally homologous (60%) and biologically related to IGF-I (Francis et al. 1989a,b). However, cIGF-II appears to exist as two variants and differs by up to 13 amino acids compared with mammalian IGF-II (Kallincos et al. 1990, Taylor et al. 1991, Upton et al. 1995). Whether these compositional differences in IGF-I and IGF-II between the species are significant to biological function or metabolism remains unknown. The role and functions of the IGFs as mediators of growth and metabolism in birds is poorly understood (McMurtry 1994). Moreover, the role of IGF-II is further complicated in the chicken by the absence of the type-2 IGF receptor found in mammalian species.
Recent evidence has established the presence of different forms of IGF-binding proteins (IGFBPs) in embryonic and adult chicken sera (Armstrong et al. 1989, Francis et al. 1990, Schoen et al. 1992, Goddard et al. 1993) and embryonic turkey sera (McMurtry et al. 1996). However, there is disagreement as to the major forms present. This situation may arise from the application of different analytical methods and the study of chickens of different sex, stage of development or strain. It was previously reported there is an apparent difference in the clearance of chicken and human forms of IGF-I from the circulation of broiler chickens, with the involvement of a predominant IGFBP with a molecular weight of 55 kDa (Francis et al. 1990). There is little information about the distribution of cIGF-II in the circulation or its uptake by tissues from blood. In the study reported herein we investigated the tissue uptake of radiolabelled cIGF-I and cIGF-II and the metabolic clearance of radiolabelled cIGF-I and cIGF-II, human IGF-I (hIGF-I) and hIGF-II in broiler chickens. One aim of the work reported herein was to evaluate whether the amino acid sequence changes between the different IGFs altered their interaction with IGFBPs in the circulation, resulting in different clearance rates from the circulation into tissues. In addition, the distribution of the radiolabelled growth factors in the plasma IGFBP pool were assessed by size-exclusion chromatography. The implications of these results for the role of the IGFs in chicken growth and metabolism are discussed.

Materials and Methods

Animals
All animal studies were conducted with research protocols approved by the Beltsville Agricultural Research Center Poultry Care Committee. Day-old male broiler chicks were purchased from Indian River (Nagadoches, TX, USA) and grown in brooder batteries (Petersime) until 4 weeks of age. A standard commercial starter diet and water were available ad libitum. At 4 weeks of age the birds were transferred to grow-out batteries and fed a commercial grower diet for the duration of the experiment. At 6 weeks of age birds that were used in the study were transferred to individual sampling cages for acclimatization. One week later (1·8–2·0 kg body weight) a dual cannula (Braintree Scientific, Inc., Braintree, MA, USA) was surgically inserted in the jugular vein as previously described (McMurtry & Brocht 1984). The infusion studies were conducted 3 days after surgery. During the 3-day post-operative period feed intake and body weight were monitored daily. Cannulated birds which did not eat or lost body weight were removed from the study.

Preparation of $^{125}$I-labelled growth factors
Recombinant cIGF-II was produced as previously described (Upton et al. 1995). The iodination of the growth factors was carried out as previously described by the chloramine-T method (Francis et al. 1990) with minor modifications. Aliquots of 25 µg of each peptide were initially dissolved in 10 µl 10 mm HCl. The mixture was neutralized with 25 µl sodium phosphate buffer (500 mmol/l), pH 7.4, to which was added 1·5 mCi carrier-free Na$^{125}$I (100 mCi/ml; Amersham Corp., Arlington Heights, IL, USA). The iodination reaction products were resolved by chromatography on Sephadex G-50 (Pharmacia LKB Biotechnology, Piscataway, NJ, USA) in 0·7 × 50 cm glass columns (Kontes, Vineland, NJ, USA) pre-equilibrated with sodium phosphate buffer (50 mmol/l), pH 7.4, containing 0·1% (v/v) chicken serum. All four growth factors were iodinated to a specific activity of 60—74 Ci/g by this method. Following chromatography, an aliquot from the $^{125}$I-labelled peptide peak was removed and trichloroacetic acid (TCA) precipitability determined. In experiment 1 the TCA precipitability was determined to be 97·9% and 98·3% for $^{125}$I-labelled cIGF-I and $^{125}$I-labelled cIGF-II respectively. In experiment 2 the TCA precipitability was determined to be 94·3%, 95·5%, 96·5% and 95·4% for $^{125}$I-labelled cIGF-I, -cIGF-II, -hIGF-I and -hIGF-II respectively.

Clearance and plasma distribution of radiolabelled cIGF-I and -II

Experiment 1 A blood sample was drawn from each bird prior to the infusion of radiolabelled hormone (time 0). Radiolabelled peptide (40 µCi/kg body weight) in a volume of 1 ml sterile saline was infused via the dual cannula. Blood samples (1 ml) were drawn into EDTA-treated tubes via the cannula at the following times post-infusion: 7·5, 15, 30, 60 and 120 min. The plasma was harvested by centrifugation (2000 g), and the radioactivity in a 10 µl aliquot determined by gamma spectrometry. TCA-precipitable radioactivity in each plasma sample was determined by mixing the 10 µl aliquot with 1 ml 10% (w/v) TCA, followed by centrifugation (2000 g), aspiration of the supernatant and counting the precipitate. The distribution pattern of radiolabelled cIGF-I and -II in the plasma IGFBP pool was assessed by size-exclusion chromatography. A 250 µl plasma sample was extracted with an equal volume of 1,1,2-trichloro-1,2,2-trifluoromethane (Freon; AR grade; Mallinckrodt, Paris, KY, USA) and 100 µl extracted lipid-free plasma chromatographed on a Superose-12 column (HR, 10/30; Pharmacia LKB) equilibrated with a solution containing sodium phosphate (50 mmol/l), sodium chloride (150 mmol/l), 0·02% sodium azide and heparin (1 U/ml) at pH 7·4. The flow rate was 0·5 ml/min and 0·5 ml fractions were taken for the measurement of radioactivity. The Superose-12
column was calibrated using the following molecular markers purchased from Pharmacia LKB Biotechnology or Sigma Chemical Co., St Louis, MO, USA: blue dextran, amylase (kDa=200), aldolase (kDa=158), ovalbumin (kDa=43), carbonic anhydrase (kDa=29), cytochrome C (kDa=12-4), aprotinin (kDa=6-5), and radiolabelled cIGF-I (kDa=7-738) and -II (kDa=7-513).

**Tissue uptake and distribution of radiolabelled growth factors**

In order to investigate tissue distribution and uptake of radiolabelled cIGF-I and -II, four birds were killed with sodium pentobarbital at 15 min and 2 h post-infusion from both treatment groups, except that n=3 birds at 2 h post-infusion in the $^{125}$I-labelled cIGF-I group. Immediately following euthanasia, the birds were exsanguinated and the following tissues were removed, weight and a 0-1-0-5 g sample taken, frozen in liquid nitrogen and counted in a single well gamma counter: brain, bursa of Fabricius, growth plate cartilage (tibiotarsus), leg muscle (muscle gastrocnemius), breast muscle (muscle pectoralis superficialis), kidney, heart, liver, pancreas, testis and small intestine. Radioactivity in faecal samples collected during the sampling period were also monitored. TCA-precipitable radioactivity in the tissue samples was determined by homogenizing (Omini tissue pulverizer; Omni International, Waterbury, CT, USA) the sample in 10% TCA (w/v), cooling on ice for 30 min and centrifuging (2000 g) to obtain TCA-soluble and TCA-insoluble fractions. The radioactivity was measured in each sample. Radioactivity in the pellets was determined and the data expressed as TCA-precipitable c.p.m./mg tissue.

**Clearance of radiolabelled cIGF-I, -II, hIGF-I and -II**

**Experiment 2** The protocol for this experiment was similar to experiment 1 except that human as well as chicken radiolabelled peptides were infused (40 µCi/kg body weight). Blood samples (1 ml) were taken at the following intervals post-infusion: 0, 0-125, 0-25, 0-5, 1, 2, 4, 6, 8, 10, 12 and 24 h. Plasma samples were processed as described in Experiment 1.

**Ligand blots**

Plasma samples collected at time 0 and pooled fractions from plasma samples subjected to Superose-12 chromatography were incubated at 60 °C for 10 min with SDSloading buffer and subjected to discontinuous SDS-PAGE through a 4% stacking gel and a 10-5% separating gel. Following electrophoresis, proteins were transferred to 0-2 µm pore size nitrocellulose sheets (BioRad Laboratories, Hercules, CA, USA) using a Trans-Blot SD Semi-Dry Transfer Cell (BioRad). The nitrocellulose sheets were air-dried and IGF ligand blots were essentially developed as described by Hossenlopp et al. (1986).

**Statistical analysis**

The TCA-insoluble radioactivity in sequential samples (experiment 2) were fitted to a tri-exponential equation with the aid of a non-linear curve fitting programme which fits non-linear regression models by least squares (Tablecurve 2D, V3-0, 32 bit; Jandel Scientific, Corte Madera, CA, USA). Pharmacokinetic parameters for each bird were determined as previously described (Ballard et al. 1991, Bastian et al. 1993). Tissue uptake data were analyzed by two-way ANOVA with replications, where $P<0.05$ was considered significant. For the pharmacokinetic study, one-way ANOVA was used and treatment means were compared using the Tukey-Kramer multiple comparisons test. Analyses were carried out using the statistical programs provided by STATSOFT, Tulsa, OK, USA. Values are expressed as means ± s.e.m. for the number of animals indicated.

**Results**

**Tissue distribution of radiolabelled growth factors**

To evaluate the transport of labelled IGFs from the circulation into tissues, the amount of $^{125}$I-labelled cIGF-I and -II in tissues 15 min and 2 h after infusion of the tracers has been expressed as TCA-insoluble radioactivity per mg tissue (Fig. 1). No correction was made for the plasma content of the different tissues. The amounts of cIGF-I and -II radioactivity per mg wet weight in the various organs or tissues are presented in rank order of distribution: kidney > testis > heart > liver > pancreas > small intestine > cartilage > bursa > gizzard > leg muscle > breast muscle > brain. Radioactivity appeared in the kidneys in a greater amount ($P<0.05$) than in any other tissue. The uptake of radiolabelled cIGF-II by the liver exceeded that of cIGF-I ($P<0.05$) at 15 min post-infusion. At 2 h post-infusion uptake of both radiolabelled peptides was similar in all tissues. The amount of radioactivity for both growth factors was significantly greater ($P<0.05$) at 15 min compared with that at 2 h, except for the leg and breast muscle, in which the amount of specific radioactivity was similar at 15 min and 2 h. The greatest decline in specific activity occurred in the kidney. This reflected the significant amounts of radioactivity detected in the faeces at 2 h (data not shown) for both radiolabelled peptides. In general, the pattern of tissue uptake and distribution did not deviate from that presented in Fig. 1 as c.p.m./mg wet tissue weight, when TCA-insoluble radioactivity was expressed as a percentage of total counts in individual tissue samples (data not shown).
Figure 1 Radioactivity in organs/tissues (c.p.m./mg TCA precipitable (ppt)) at 15 min (cIGF-I (solid bars) and cIGF-II (open bars)) and at 2 h (cIGF-I (cross-hatched bars) and cIGF-II (shaded bars)) after the administration of radiolabelled tracers. Values are means ± S.E.M. for three birds for cIGF-I and four birds for cIGF-II.

Table 1 Pharmacokinetic parameters for radiolabelled IGF peptides administered to chickens as determined by TCA-precipitable radioactivity. Half-life of bound and free \(^{125}\)I-labelled IGF peptides were calculated from a three-component curve. Values are means ± S.E.M.; \(n = 11\)

<table>
<thead>
<tr>
<th>Labelled peptide</th>
<th>MCR (ml/min per kg)</th>
<th>Component half-life (min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>cIGF-I</td>
<td>1-88 ± 0-39^a</td>
<td>5-66 ± 0-29^a</td>
<td>49-61 ± 2-99^a</td>
<td>486-67 ± 19-64^a</td>
<td></td>
</tr>
<tr>
<td>cIGF-II</td>
<td>3-33 ± 0-08^b</td>
<td>3-11 ± 0-11^b</td>
<td>36-58 ± 1-14^b</td>
<td>444-11 ± 32-50^b</td>
<td></td>
</tr>
<tr>
<td>hIGF-I</td>
<td>2-94 ± 0-13^c</td>
<td>4-45 ± 0-17^c</td>
<td>42-63 ± 2-89^c</td>
<td>453-44 ± 27-02^c</td>
<td></td>
</tr>
<tr>
<td>hIGF-II</td>
<td>3-45 ± 0-10^b</td>
<td>3-01 ± 0-36^b</td>
<td>35-02 ± 3-27^b</td>
<td>413-61 ± 29-18^b</td>
<td></td>
</tr>
</tbody>
</table>

Mean values in a column with the same superscript are not significantly different (\(P > 0.05\)).

MCR, metabolic clearance rate.

Profiles of growth factor radioactivity in plasma

Chromatography of radiolabelled cIGF-I, cIGF-II, hIGF-I and hIGF-II at neutral pH on a Superose-12 column produced multiple peaks of radioactivity (Figs 2 and 3). For brevity, only the chromatography results of experiment 2 will be discussed. The patterns of change in radioactivity of \(^{125}\)I-labelled cIGF-I and \(^{125}\)I-labelled cIGF-II were similar in experiments 1 and 2. Chromatography of plasma samples taken at 7-5 min post-infusion of all four radio-labelled peptides revealed three peaks of radioactivity at 150 kDa, 43 kDa and free peptide (Figs 2 and 3). A fourth
Figure 2 Superose-12 column neutral chromatography of plasma from chickens following a bolus injection of (a) $^{125}$I-labelled cIGF-I and (b) $^{125}$I-labelled cIGF-II. Values represent mean values of four birds with a pooled S.E.M. ± 17 c.p.m. for cIGF-I and four birds with a pooled S.E.M. ± 18 c.p.m. for cIGF-II. Chickens were sampled at 7.5 min (□), 15 min (○), 30 min (●), 60 min (▼), 2 h (▲), 4 h (+), 6 h (×), 8 h (*), 10 h (–), 12 h (■) and 24 h (■). The positions of molecular mass markers used to calibrate the column are indicated: D, dextran; A, aldolase; O, ovalbumin; I, $^{125}$I-labelled cIGF-I; II, $^{125}$I-labelled cIGF-II.

The peak of radioactivity corresponding to low molecular weight degradation products was evident at 15 min post-infusion and its proportion of the total radioactivity increased with time (data not shown). At 7.5 min post-infusion, a majority (53%) of the $^{125}$I-labelled cIGF-I radioactivity was found in the 43 kDa fractions (31–34; Fig. 2a). At this time-point, 37% of the radioactivity was free peptide (fractions 36–39), while the remaining 10% was associated with the 150 kDa fraction (27–29). With time, the radioactivity associated with the three peaks...
changed in such a manner that by 2 h post-infusion the majority of the protein-associated radioactivity was in the 150 kDa component. This pattern remained constant such that by 6 h post-infusion the majority (98%) of the residual radioactivity was associated with the large molecular mass component. Only negligible amounts of radioactivity were noted in the samples collected after 8 h. The general pattern of radioactivity distribution between the various components was similar between 125I-labelled cIGF-I (Fig. 2a) and 125I-labelled hIGF-I (Fig. 3a).

A slightly different pattern of distribution of radioactivity was observed following neutral chromatography of...
Table 2 Half-life of bound and free radio-labelled peptides calculated from the three components determined by Superose-12 chromatography. Values are means ± S.E.M.; n = 11

<table>
<thead>
<tr>
<th>Labelled peptide</th>
<th>Free peptide component (min)</th>
<th>43 kDa peptide component (min)</th>
<th>150 kDa peptide component (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cIGF-I</td>
<td>5.17 ± 0.27</td>
<td>55.84 ± 4.20</td>
<td>469.03 ± 23.66</td>
</tr>
<tr>
<td>cIGF-II</td>
<td>2.76 ± 0.44</td>
<td>39.73 ± 2.01</td>
<td>439.11 ± 30.93</td>
</tr>
<tr>
<td>hIGF-I</td>
<td>4.09 ± 0.28</td>
<td>44.66 ± 1.89</td>
<td>453.45 ± 46.80</td>
</tr>
<tr>
<td>hIGF-II</td>
<td>3.07 ± 0.57</td>
<td>37.32 ± 2.62</td>
<td>448.67 ± 39.84</td>
</tr>
</tbody>
</table>

Mean values in a column with the same superscript are not significantly different (P>0.05).
Free peptide component = 125I-associated radioactivity in fractions 36–40; 43 kDa component = that in fractions 31–35; 150 kDa component = that in fractions 26–30 (see Figs 2 and 3).

125I-labelled cIGF-II (Fig. 2b) and 125I-labelled hIGF-II (Fig. 3b). The same three major peaks of radioactivity that were observed to be associated with radiolabelled cIGF-I and hIGF-I were noted with the radiolabelled IGF-II peptides. However, with both radiolabelled IGF-II peptides a shoulder on the descending part of the 150 kDa component (fractions 29–30) was observed. The shoulder (fractions 29–30) on the descending part of the 150 kDa radioactivity curve (Fig. 2b and Fig. 3b) is attributed to the presence of a 70 kDa IGBP. This IGBP-like protein was detected by ligand blotting when radiolabelled cIGF-II and hIGF-II were used as probes, but no cIGF-I or hIGF-I (Fig. 4). With both radiolabelled IGF-II peptides the amount of radioactivity in these fractions was reduced (note fraction 30; Fig. 2a and Fig. 3a), while more radioactivity was counted in these fractions following neutral fractionation of 125I-labelled cIGF-II and 125I-labelled hIGF-II (Fig. 2b and Fig. 3b). Despite this difference, the overall distribution pattern of the radiolabelled IGF-II peptides was similar to that observed for cIGF-I and hIGF-I.

Clearance of growth factor radioactivity from plasma

Pharmacokinetic analysis of the data demonstrated differences in the clearance of the four radio-labelled peptides. As previously described, half-life estimates were calculated from TCA-precipitable radioactivity present in each plasma sample (Table 1). Statistical analysis of the data revealed that clearance could be described by a three-exponential equation. For component 1, half-lives of 125I-labelled cIGF-I (5.66 ± 0.29 min) and 125I-labelled hIGF-I (4.45 ± 0.17 min) were significantly (P<0.05) different. Both IGF-II species (125I-labelled cIGF-II=3.11 ± 0.11 min and 125I-labelled hIGF-II= 3.01 ± 0.36 min) were cleared at a rate greater (P<0.05) than either IGF-I. The clearance of the radiolabelled growth factors associated with component 2 followed a similar pattern (Table 1). No difference was observed in the metabolism of the radiolabelled growth factors associated with component 3.

The clearance of the three pools of radiolabelled IGFs resolved by neutral chromatography are presented in Table 2. A metabolic clearance pattern similar to the TCA-insoluble calculations was evident when total chromatographically resolved radioactivity in the three pools was used to calculate clearance parameters (Table 2). Statistical analysis revealed that clearance rates between the four radio-labelled peptides in the three pools (free peptide, 43 kDa component, and 150 kDa component) were significantly (P<0.05) different. Free radiolabelled cIGF-II and hIGF-II were metabolized at a greater rate (half-life=2.76 ± 0.44 and 3.07 ± 0.57 min respectively) compared with cIGF-I (3.17 ± 0.27 min) and hIGF-I (4.09 ± 0.28 min). The clearance rate of hIGF-I exceeded that of cIGF-I (P<0.05). A similar pattern of clearance was noted in the radiolabelled growth factors associated with the 43 kDa component. The half-life of 125I-labelled cIGF-I (55.84 ± 4.20 min) was greater than hIGF-I (44.66 ± 1.89 min). Both radiolabelled IGF-IIs were cleared at a greater rate than their IGF-I counterparts (cIGF-II=39.73 ± 2.01; hIGF-I=37.32 ± 2.67). There was no apparent difference in the clearance of tracer complexed to the 150 kDa component (Table 2). It was apparent that radiolabelled IGF-II peptides (free tracer) were metabolized at a greater rate than either IGF-I peptide factor. This was substantiated by the observation that the metabolic clearance rate (ml/min per kg) of the IGF-II growth factors was greater than either IGF-I peptide (Table 1).

Analysis of IGFBPs associated with radioactive growth factors

To compare the binding of the four radioligands to serum IGFBPs, a chicken plasma pool sample was subjected to ligand blot analysis. For comparative purposes, a porcine plasma sample was electrophoresed and blotted along with the chicken sample. The autoradiographic profile of these samples is shown after probing with radiolabelled cIGF-I, cIGF-II, hIGF-I or hIGF-II (Fig. 4). When chicken plasma was probed with cIGF-I or hIGF-I, a major band...
Figure 4 Ligand blotting of chicken plasma (C) and porcine serum (P) IGFBPs. Samples were subjected to gel electrophoresis, blotted and probed with \(^{125}\text{I-}\)labelled cIGF-I, \(^{125}\text{I-}\)labelled cIGF-II, \(^{125}\text{I-}\)labelled hIGF-I or \(^{125}\text{I-}\)labelled hIGF-II (indicated by asterisk). The molecular weight standards in 5 mmol dithiothreitol/ml (Sigma Chemical Co.) were: \(\beta\)-galactosidase (116 kDa), transferrin (78 kDa), BSA (68 kDa), ovalbumin (43 kDa) and carbonic anhydrase (29 kDa). Nitrocellulose paper was exposed to autoradiographic film for 10 days.

at 33 kDa was evident and a minor single band was present at 42 kDa. Conversely, pig plasma probed with the same radioligands revealed a doublet band at 42–43 kDa and a minor band at 33 kDa.

A different pattern of IGFBP activity was observed when the blood samples were probed with either \(^{125}\text{I-}\)labelled cIGF-II or \(^{125}\text{I-}\)labelled hIGF-II. The intensity and position of the bands at 33 and 42 kDa were similar to that observed when radiolabelled cIGF-I or hIGF-I were used. However, an additional major band with a molecular mass of 70 kDa was present when radiolabelled cIGF-II or hIGF-II were employed as probe. The 70 kDa protein was only present in chicken plasma as this band was not found to be present in pig plasma (Fig. 4). Also, as noted by the increased amount of plasma required for blotting (4.0 vs 0.4 µl for the chicken and pig respectively), it was apparent that the concentration of IGFBP activity was clearly greater in pig plasma compared with chicken plasma. From this study it cannot be determined whether these differences are due to changes in affinities for the IGFs or absolute concentration differences of IGFBPs.

The presence of the 70 kDa binding protein was demonstrated by the autoradiographic results obtained from plasma samples separated by neutral chromatography (Fig. 5). In this experiment, plasma collected from birds prior to the infusion of radiolabelled peptides (time 0) were pooled, chromatographed at neutral pH and the fractions subjected to ligand blotting (details in Fig. 5). Results of this experiment illustrate the presence of the 70 kDa protein only when \(^{125}\text{I-}\)labelled cIGF-II was used as probe (lanes 3 and 4 and 7 and 8). The neutral chromatography fraction equivalents of the various lanes are presented in Fig. 5. These data also indicated that the IGFBP at 42–43 kDa must be part of the high molecular mass binding protein complex, analogous to that observed in mammalian systems.

Discussion

hIGF-I has been shown to influence various aspects of metabolism, differentiation and proliferation of cells derived from embryonic and post-hatch chicks in vitro (Vasilatos-Younken & Scanes 1991, Duclos et al. 1993). Few studies have reported using cIGF-I because of insufficient quantities of the peptide. Whether IGF-II (human or chicken) behaves similarly is unknown. The relative physiological and biochemical functions attributed to IGF-I and -II in birds are confounded by the fact that the type-2 IGF receptor is absent (Kasuga et al. 1982, Bassas et al. 1988, Duclos & Goddard 1990), unlike the situation in mammals (Rechler & Nisssly 1983). In an earlier report (Francis et al. 1990) it was demonstrated that radiolabelled hIGF-I administered in vivo in adult chickens is cleared more rapidly from the circulation than native cIGF-I. In the study reported herein, we have expanded the previously mentioned investigation (Francis et al. 1990) to gain a more comprehensive understanding of the metabolism of IGFs and identify which organ/tissue functions may be influenced by the IGFs in birds.
This study also demonstrates that, in the chicken, IGF-I and -II are rapidly taken up by tissues. The pattern of tissue distribution is similar to that reported in rats (Ballard et al. 1991, Bastian et al. 1993) with the greatest uptake of both radiolabelled peptides occurring in the kidney. It has been previously shown that the kidney has an important role in the turnover of insulin in the chicken, most likely involving specific insulin receptors (Milton et al. 1985). Whether uptake of the radiolabelled peptides by the kidney in the chicken involves specific IGF-I receptors or insulin receptors remains speculative in view of the fact that few studies have reported tissue or organ IGF receptor distribution in birds. However, kidney IGF-I receptors have been reported for the rat, monkey and pig (Rechler & Nisley 1983).

The uptake of both radiolabelled peptides by the testes suggests that the IGFs may be involved in testicular function in birds. Although IGF-I receptors have not been reported for avian reproductive tissues, specific IGF-I receptors are known to be present in comparative tissues in mammals (Chatelain et al. 1987).

The only tissue in which cIGF-II uptake exceeded \( (P<0.05) \) IGF-I was noted in the liver at 15 min post-infusion, similar to the situation in the rat (Ballard et al. 1991). In the latter species the preferential uptake of IGF-II can be explained by the presence of the type-2 receptor. Type-1 receptors have been described in the avian liver (Bassas et al. 1988, Duclos & Goddard 1990), suggesting that the uptake of radiolabelled IGF-II is associated with the IGF-I receptors in this tissue. Indeed, except for the liver, the similarity in uptake between cIGF-I and -II by all of the other tissues examined strongly suggests that a majority of the uptake can be attributed to association with the type-1 receptor. The basis for the preferential uptake of IGF-II over IGF-I in the liver is not clear.

Uptake of significant amounts of both radiolabelled cIGFs by the pancreas strongly suggests that the IGFs may modulate pancreatic secretory functions in birds as has been reported for mammals (Van Schravendijk et al. 1987, Guler et al. 1989). Similarly, the uptake of radioactivity present by the growth plate cartilage is indicative that this tissue is a target tissue for the IGFs. This is substantiated by recent reports (Rosselot et al. 1992, 1994) in which it was demonstrated that avian growth plate chondrocytes are responsive in vitro to IGF-I and not growth hormone, unlike the situation in mammals (Ohlsson et al. 1992).

At this stage the interaction between the IGFs and immune function in birds has not been investigated. In birds, the bursa is an organ attached to the dorsal wall of the protoderm which produces lymphoid cells that generate humoral antibodies (Ratcliff et al. 1987). McFarland et al. (1992) have reported in the turkey that the highest levels of specific IGF-I binding was observed in bursa of Fabricius membrane preparations compared with that in 13 other tissues surveyed. The observation in the present study that the uptake of radioactive tracer by bursa of Fabricius suggests that the IGFs may also play an endocrine role in immune function.
IGF-I has previously been shown to stimulate heart mesenchymal cell proliferation (Balk et al. 1984), which in turn may account for the uptake of both peptides by the heart observed in our study. Somewhat surprising was the absence of accumulation of either tracer in skeletal muscle. Indeed, the amount of $^{125}$I-cIGF-I and -II radioactivity in leg and breast muscle samples was not different for either peptide nor was uptake different between sampling times. Specific IGF-I receptors have been identified in chick embryonic skeletal muscle (Trouten-Radford et al. 1991) as well as chicken muscle satellite cells (Duclos et al. 1991). Conversely, significant amounts of $^{125}$I-cIGF-I and -II radioactivity were detected in the gizzard, an organ comprised mainly of smooth muscle whose cells undergo significant stress due to the mechanical grinding of food. Specific IGF-I receptors have been reported to be present in this tissue in the turkey (McFarland et al. 1992).

We have confirmed our previous findings that the clearance of labelled chicken and human IGF-I are different (Tables 1 and 2). cIGF-I was cleared at a slower rate than all of the growth factors tested. hIGF-I was metabolized at a greater rate than cIGF-I, similar to that previously observed (Francis et al. 1990). This difference in clearance could be attributed to differences in the rates of clearance of unbound radiolabelled growth factor and that associated with the 43 kDa binding protein. Half-lives for hIGF-I in the chicken of 43 min (McGuinness & Cogburn 1990) and 15 min (Buonomo et al. 1987) have been reported. This is the first report of the clearance of IGF-II in the chicken. The $^{125}$I-labelled cIGF-II and $^{125}$I-labelled hIGF-II were cleared at similar rates and at rates greater than their IGF-I counterparts (Tables 1 and 2). The role of the unique IGF-II binding protein (70 kDa) in the metabolism of these peptides is unknown.

Schoen et al. (1992) have demonstrated that binding of radiolabelled IGF-I and -II to proteins present in serum increases with age in the chick, and that the binding (in vitro) is two- to threefold greater for IGF-II than for IGF-I. Their data further suggests that the increase in total binding activity is not the result of changing endogenous growth factor concentrations or affinities, but most likely reflects an increase in the amount of IGFBPs. However, this cannot be verified until specific immunonassays for the chicken IGFBPs are developed. No longitudinal studies on chicken IGFBP profiles using ligand blots as an estimate of binding protein activity have been reported.

The pattern of plasma IGFBPs observed in this study on ligand blots of 4-week-old chickens resembles that reported by Armstrong et al. (1989) and Schoen et al. (1992). Similar to Schoen et al. (1992) we also detected a 70 kDa IGFBP, which was evident only when either $^{125}$I-labelled cIGF-II or $^{125}$I-labelled hIGF-II were used as probes. This high molecular mass IGF-binding species was not observed by others in chicken serum (Armstrong et al. 1989, Lee et al. 1989, Upton et al. 1992, Lord et al. 1994), due most likely to the fact that $^{125}$I-labelled hIGF-I was employed as probe rather than radiolabelled IGF-II. More recently, the presence of this 70 kDa IGFBP in chicken serum has been confirmed (Upton et al. 1995). A similar sized IGFBP was observed to be present in embryonic turkey serum (McMurtry et al. 1996). Hodgkinson et al. (1989) have previously reported an uncharacterized IGF-II-specific IGFBP of 70–100 kDa in clearance studies of IGF-II but not IGF-I in sheep. It has been suggested that the 42 kDa IGFBP detected in ligand blots (also noted to be present in this experiment) may represent the avian equivalent of mammalian IGFBP-3 (Schoen et al. 1992, Upton et al. 1993). An IGFBP similar in molecular mass has been reported to be present in embryonic turkey serum and is apparently sensitive to the ontogeny of growth hormone secretion in this species (McMurtry et al. 1992) and chick embryo (Schoen et al. 1992). Taken together, these observations provide strong evidence that the 42 kDa binding protein is the avian analogue to mammalian IGFBP-3. There was no evidence of a high molecular mass binding species (>200 kDa) in chicken plasma as has been reported to be present in sheep blood and attributed to circulating type-2 receptor (Hodgkinson et al. 1989). The major pool of radiolabelled IGF for each peptide studied was resolved as a 43 kDa complex by chromatography, this corresponds to the main 31 kDa IGFBP on ligand blots of chicken serum. Schoen et al. (1992) identified this approximately 31 kDa IGFBP on ligand blots by immunoprecipitation as cIGFBP-2. This putative cIGFBP-2 appears to bind IGF-II better than IGF-I on ligand blots, a result that contrasts with the more rapid metabolism of the corresponding 43 kDa pool resolved by chromatography. Clearly, the dynamics of IGF exchange between pools identified in blood and the target tissues remain to be resolved. Following in vitro incubation of chicken plasma and radiolabelled hIGF-I, no large molecular mass binding activity was observed and binding was detected only in the 30–50 kDa region (Lord et al. 1994). A similar distribution pattern, i.e. the absence of any large molecular mass IGFBP (150 kDa) in chicken plasma incubated in vitro with radiolabelled IGF-I has been previously reported, whereas in vivo labelling of a 150 kDa protein was observed (Francis et al. 1990). The data presented in this study suggest that the IGF-binding properties determined in vitro do not necessarily reflect the situation in vivo.

Taken together, the results of this study demonstrate that, in the chicken, IGFs are rapidly taken up by tissues and the peptides are associated in plasma with binding proteins in a manner similar to mammals. An apparent IGFBP-like binding protein (70 kDa) is present in chicken blood which appears to be specifically associated with IGF-II in the avian species. Whether this binding protein functions and has the same attributes as the various IGFBPs described for mammals remains to be elucidated.
Acknowledgements

The technical assistance of Debbie Gavelek and Shirley Lutz is greatly appreciated. We wish to thank John Ballard for stimulating discussions that supported our research. Support to ZU and GLF from the Chicken Meat Research Council of Australia is acknowledged. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other suitable products.

References


Balk SD, Moris A, Gunther HS, Svoboda MF, Van Wyk JJ, Nisley SP & Scares CG 1984 Somatomedins (insulin-like growth factors), but not growth hormone, are mitogenic for chicken heart mesenchymal cells and act synergistically with epidermal growth factor and brain fibroblast growth factor. Life Science 34 335–346.


Buonomo FC, Lautero TJ, Baile CA & Daughaday WH 1987 Effect of insulin-like growth factor I (IGF-I) on growth hormone-releasing factor (GRF) and thyrotropin-releasing hormone (TRH) stimulation of growth hormone (GH) secretion in the domestic fowl (Gallus domesticus). General and Comparative Endocrinology 66 274–279.


Fawcett DH & Bulfield G 1990 Molecular cloning, sequence analysis and expression of putative chicken insulin-like growth factor-I cDNAs. Journal of Molecular Endocrinology 4 201–211.


Received 13 September 1995
Revised manuscript received 14 February 1996
Accepted 26 March 1996