Formation of complexes between ^{125}I -labelled human or bovine somatotropins and binding proteins *in vivo* in rat liver and kidney

Juan S. BONIFACINO,* Leonor P. ROGUIN and Alejandro C. PALADINI

Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires and Centro para el Estudio de las Hormonas Hipofisarias, Junín 956, 1113 Buenos Aires, Argentina

(Received 31 January 1983/Accepted 14 March 1983)

At 5 min after intravenous injection, both ^{125}I -labelled human somatotropin and ^{125}I -labelled bovine somatotropin were concentrated in rat liver and kidney. When the labelled hormones were administered along with an excess of the corresponding unlabelled hormone, a significant decrease of the uptake was observed in the liver, but not in the kidney. Study of the subcellular distribution of radioiodinated somatotropins in liver revealed that most of the radioactivity was specifically concentrated in the microsomal fraction. In contrast, the kidney fraction that accounted for most of the radioactivity was the 100 000 g supernatant. After solubilization, with 1% (w/v) Triton X-100, of the microsomal fractions obtained from both organs, the radioactive material was analysed by gel filtration on Sepharose CL-6B. By using this approach, it was demonstrated that both ^{125}I -labelled human somatotropin and ^{125}I -labelled bovine somatotropin bind *in vivo* to proteins present in liver. A small proportion of ^{125}I -labelled human somatotropin was also shown to form complexes with proteins present in kidney. The present results demonstrate that the liver uptake is mainly due to binding of somatotropins to specific proteins, in contrast with the kidney, in which binding to specific sites contributes minimally to the overall uptake.

The liver and the kidneys are the organs where the highest concentration of radioactivity occurs after injection of labelled growth or lactogenic hormones into animals of different species (Birkinshaw & Falconer, 1972; Retegui-Sardou *et al.*, 1977; Johnson & Maack, 1977). The uptake of these hormones seems to be the result of different processes in each organ. It has been demonstrated that the uptake of ^{125}I -labelled human somatotropin into rat liver is decreased by the simultaneous injection of excess unlabelled human somatotropin (Turyn & Dellacha, 1978; Durán-García *et al.*, 1981), whereas the uptake into kidney remains unaffected. The uptake of ^{125}I -labelled bovine somatotropin or ^{125}I -labelled sheep prolactin into rat liver have also been shown to be decreased by concomitant administration of the respective unlabelled hormones (Turyn & Dellacha, 1978; Durán-García *et al.*, 1981). Hence it has been suggested that liver uptake, in contrast with kidney uptake, responds to a saturable process.

* Present address: Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205, U.S.A.

The saturability of the uptake of growth and lactogenic hormones into liver could arise from the existence of specific receptors mediating the process. Binding sites for both kinds of hormones have been demonstrated *in vitro* in the liver of animals from various species (Posner *et al.*, 1974*a,b*; Herington *et al.*, 1976*a,b*; Turyn *et al.*, 1976; Etzrodt *et al.*, 1976; Postel-Vinay, 1976; Ranke *et al.*, 1976*a,b*; Postel-Vinay & Desbuquois, 1977; Bonifacino *et al.*, 1978, 1981; Silverstein & Richards, 1979; Mountjoy *et al.*, 1980; Baxter *et al.*, 1980). However, their role in the uptake of the respective hormones *in vivo* remains yet to be established.

The present study was made in an attempt to gain some insight into the nature of the uptake processes occurring in liver and kidney. To this end, we investigated the subcellular distribution of ^{125}I -labelled human and bovine somatotropins in both organs, after the injection of these hormones *in vivo*. Furthermore, the radioactive material associated with the microsomal fraction was analysed by gel filtration, after solubilization with Triton X-100. These experiments enabled us to demonstrate that the formation of hormone-binding-protein complexes *in vivo* is basically responsible for the

saturable uptake observed in liver. In contrast, this phenomenon, when present, contributes very little to the uptake in the kidney.

Materials and methods

Materials

The hormones and chemicals used in this work were prepared or obtained as indicated previously (Bonifacino *et al.*, 1981; Roguin *et al.*, 1982).

Iodination of hormones

Human somatotropin (3.0 i.u./mg) was radioiodinated and purified as described by Bonifacino *et al.* (1981). The same procedures were employed to prepare ^{125}I -labelled bovine somatotropin. Their specific radioactivities ranged from 86 to 124 $\mu\text{Ci}/\mu\text{g}$ and from 70 to 94 $\mu\text{Ci}/\mu\text{g}$ respectively. The radio-labelled hormones were homogeneous, as analysed by gel filtration on Sepharose CL-6B by the method described below. In both cases only one peak of radioactivity corresponding to the monomeric species was observed (K_D 0.59). The radioiodinated somatotropins were used up to 3 days after labelling and then were discarded. Radioactivity was measured with a gamma-radiation scintillation counter with an efficiency of 25%.

Experiments performed *in vivo*

Adult female or male rats of the Long-Evans strain (200–280 g) were injected intravenously via the tail vein, under light ether anaesthesia, with 300–450 ng of ^{125}I -labelled human somatotropin or ^{125}I -labelled bovine somatotropin (2×10^7 – 3×10^7 c.p.m.) contained in 0.24–0.70 ml of 0.1 M-NaCl/1% (w/v) bovine serum albumin/50 mM-potassium phosphate buffer, pH 7.4. Alternatively, unlabelled human somatotropin or bovine somatotropin (1.2 mg) was injected along with the labelled hormone. After 5 min, the animals were killed by

cervical dislocation. Triplicate samples of blood (0.1–0.2 ml each) were extracted by heart puncture, and immediately the liver and kidneys were removed, rinsed with cold saline (0.9% NaCl), weighed and homogenized with 10 vol. (v/w) of cold 0.3 M-sucrose/0.5 mM- CaCl_2 /5 mM-Tris/HCl buffer, pH 7.5, in a glass homogenizer with a Teflon pestle. A 0.5 ml sample of the homogenate was taken for counting of the radioactivity. The uptake was expressed as the ratio of (c.p.m./g) organ to (c.p.m./g) blood. The rest of the homogenate was subjected to the fractionation procedure described in Scheme 1. Liver and kidneys rendered 34 and 14 mg of protein in the microsomal fractions per g of organ respectively. Approx. 30 mg of protein of each microsomal fraction was resuspended in 3 ml of 1% (w/v) Triton X-100/25 mM-Tris/HCl buffer, pH 7.5. The suspension was centrifuged at 100 000 g for 1 h (r_{av} 5.4 cm). A thin layer of lipids on the surface of the supernatant was removed by aspiration and discarded. The supernatant ('solubilized microsomes') and the pellet ('insoluble microsomes') were separated by decantation. The whole procedure was performed at 4°C. Triplicate samples of each subcellular fraction were taken for counting of the radioactivity. The overall recovery of radioactivity after the fractionation procedure ranged from 92 to 100%. The radioactive material present in the 100 000 g supernatants and in the detergent-solubilized microsomes was analysed by gel filtration on Sepharose CL-6B.

Experiments performed *in vitro*

Complexes between ^{125}I -labelled human somatotropin and binding proteins from female or male rat liver were formed *in vitro* by two different procedures.

(a) Liver microsomal fractions from normal rats were prepared in accordance with Scheme 1. The microsomal fractions (30 mg of protein) were in-

Scheme 1. Fractionation procedure employed for liver or kidney homogenates
For full experimental details see the text.

cubated for 16 h (overnight) at 25°C with ^{125}I -labelled human somatotropin (2.2×10^6 c.p.m.) in 10 mM- CaCl_2 /0.08% bovine serum albumin/25 mM-Tris/HCl buffer, pH 7.5, in a total volume of 1.5 ml with or without 2 mg of unlabelled human somatotropin. After incubation, the reaction mixtures were centrifuged at 100 000 g (r_{av} , 5.4 cm) for 1 h at 4°C, and the radioactivities in the pellets were counted. Under these conditions specific binding ranged from 32.2 to 40.2% for female rats and from 1.1 to 4.3% for male rats. The pellets were solubilized as described above for the experiments performed *in vivo*.

(b) Liver microsomal fractions from normal rats were solubilized with 1% (w/v) Triton X-100 as described previously (Bonifacino *et al.*, 1981). Detergent-solubilized extracts (0.12 mg of protein) were incubated for 6 h at 25°C with ^{125}I -labelled human somatotropin (2.22×10^5 c.p.m.) in 0.5 ml of the same incubation medium cited in (a) with or without 5 μg of unlabelled human somatotropin. Under these conditions specific binding ranged from 11.1 to 13.1% for female rats and from 0.8 to 2.7% for male rats, as determined by the poly(ethylene glycol) precipitation method (Bonifacino *et al.*, 1981).

Samples obtained by procedures (a) and (b) were analysed by Sepharose CL-6B chromatography.

Gel-filtration chromatography

Gel-filtration chromatography of the 100 000 g supernatants labelled *in vivo* and the detergent-solubilized microsomes labelled *in vivo* or *in vitro* was performed on a Sepharose CL-6B column (1.6 cm $\times$ 63.1 cm) equilibrated and eluted at 4°C with 10 mM- CaCl_2 /0.1% (w/v) Triton X-100/0.02% NaN_3 /25 mM-Tris/HCl buffer, pH 7.5. Fractions of volume approx. 1.4 ml were collected at a flow rate

of 18 ml/h and their radioactivities were counted. The column was previously calibrated with standard proteins as described elsewhere (Roguin *et al.*, 1982). The equation of the calibration curve employed to calculate Stokes radii (a) from distribution coefficients (K_D) was:

$$K_D^{\frac{1}{3}} = -0.0039a + 0.93$$

Results

Uptake of ^{125}I -labelled human somatotropin and ^{125}I -labelled bovine somatotropin in vivo into rat liver and kidney

At 5 min after intravenous injection, both ^{125}I -labelled human somatotropin and ^{125}I -labelled bovine somatotropin were concentrated in liver and kidney of female as well as male rats (Table 1). When the radioactive somatotropins were administered along with an excess of the corresponding unlabelled hormone, the liver/blood ratios decreased, in contrast with the kidney/blood ratios, which did not show significant changes (Table 1). The greatest uptake of radioactivity in liver was found when ^{125}I -labelled human somatotropin was injected into female rats. The liver uptake of ^{125}I -labelled human somatotropin in male rats and the liver uptake of ^{125}I -labelled bovine somatotropin in rats of both sexes showed similar values (Table 1).

Subcellular distribution of ^{125}I -labelled human somatotropin and ^{125}I -labelled bovine somatotropin in rat liver and kidney

Table 2 shows that ^{125}I -labelled human somatotropin taken up into female rat liver was predominantly associated (58%) with the crude microsomal fraction. Only 8% of the radioactivity was found in the 100 000 g supernatant. The microsomal

Table 1. Uptake of ^{125}I -labelled human somatotropin and ^{125}I -labelled bovine somatotropin in vivo into rat liver and kidney

Values are means $\pm$ S.D. for determinations performed on the numbers of animals shown in parentheses. The statistical significance of the differences between the organ/blood ratios obtained in the absence and in the presence of excess of unlabelled hormone was analysed by using Student's *t* test. Probabilities are as follows: **P* < 0.02; ***P* < 0.01; ****P* < 0.001.

^{125}I -labelled somatotropin Injected	Unlabelled somatotropin injected	Sex	Uptake of labelled hormone	
			Liver/blood ratio	Kidney/blood ratio
Human	—	Female	5.4 $\pm$ 1.3 (3)	7.8 $\pm$ 0.8 (3)
Human	Human	Female	0.7 $\pm$ 0.0 (3)	6.8 $\pm$ 1.0 (3)
Human	—	Male	2.6 $\pm$ 0.8 (3)	11.5 $\pm$ 0.7 (3)
Human	Human	Male	0.5 $\pm$ 0.1 (3)	10.1 $\pm$ 1.0 (3)
Bovine	—	Female	1.7 $\pm$ 0.4 (4)	4.7 $\pm$ 1.5 (4)
Bovine	Bovine	Female	0.6 $\pm$ 0.3 (4)	6.7 $\pm$ 5.0 (4)
Bovine	—	Male	1.7 $\pm$ 0.2 (3)	7.2 $\pm$ 2.8 (3)
Bovine	Bovine	Male	0.5 $\pm$ 0.1 (3)	6.5 $\pm$ 1.1 (3)

fraction showed the lowest content of protein among all the fractions derived from the homogenate (detailed results not shown). Thus this fraction represents the major site of concentration of the hormone in liver. The injection of an excess of unlabelled human somatotropin together with the radioiodinated hormone led to a change in the distribution pattern (Table 2). The proportion of radioactivity in the 100 000 g supernatant increased to 40%, whereas the proportion of radioactivity in the microsomal fraction decreased to 38%.

Unlike the situation in the liver, the kidney fraction that accounted for most of ^{125}I -labelled human somatotropin (33%) was the 100 000 g supernatant. The microsomal fraction showed the lowest proportion (12%) of radioiodinated hormone in this organ (Table 2). None of the subcellular fractions concentrated ^{125}I -labelled human somatotropin predominantly when the amount of proteins recovered in each fraction was taken into account (detailed results not shown). The relative distri-

bution of the labelled hormone among the different fractions remained unchanged by the simultaneous injection of unlabelled human somatotropin (Table 2).

In order to check that the subcellular distribution of the labelled hormone in liver and kidney had not been generated as an artifact during homogenization and fractionation, ^{125}I -labelled human somatotropin (1×10^6 c.p.m.) was added to 30 ml of liver homogenate or kidney homogenate and the samples were processed in a way similar to that employed in the experiments performed *in vivo*. Under these conditions, over 90% of radioactivity was observed in the 100 000 g supernatant of both organs, confirming that the distribution is not due to an artifact (detailed results not shown).

Similar conclusions were drawn from the study of the subcellular distribution of ^{125}I -labelled human somatotropin in male liver and kidney and from that of ^{125}I -labelled bovine somatotropin in female or male liver and kidney (Tables 2 and 3).

Table 2. Subcellular distribution of radioactivity in rat liver and kidney 5 min after the injection of ^{125}I -labelled human somatotropin into female or male rats

Symbols (—) and (+) indicate the absence or the presence of excess of unlabelled human somatotropin in the injected solution respectively. Values are means $\pm$ S.D. for determinations performed on three animals.

Fraction	Relative distribution of radioactivity (%)							
	Female				Male			
	Liver		Kidney		Liver		Kidney	
	—	+	—	+	—	+	—	+
Nuclear	26 $\pm$ 8	14 $\pm$ 5	31 $\pm$ 12	27 $\pm$ 7	24 $\pm$ 1	16 $\pm$ 6	26 $\pm$ 2	23 $\pm$ 1
Mitochondrial	8 $\pm$ 3	8 $\pm$ 8	24 $\pm$ 3	28 $\pm$ 2	6 $\pm$ 3	11 $\pm$ 2	22 $\pm$ 4	24 $\pm$ 1
Microsomal	58 $\pm$ 7	38 $\pm$ 10	12 $\pm$ 1	13 $\pm$ 4	57 $\pm$ 3	30 $\pm$ 1	15 $\pm$ 1	14 $\pm$ 1
100 000 g supernatant	8 $\pm$ 1	40 $\pm$ 8	33 $\pm$ 8	32 $\pm$ 5	13 $\pm$ 2	43 $\pm$ 6	37 $\pm$ 5	39 $\pm$ 1
Solubilized microsomes	50 $\pm$ 6	32 $\pm$ 9	10 $\pm$ 1	11 $\pm$ 3	43 $\pm$ 3	30 $\pm$ 0	12 $\pm$ 1	11 $\pm$ 1
Insoluble microsomes	8 $\pm$ 0	4 $\pm$ 1	2 $\pm$ 0	2 $\pm$ 1	8 $\pm$ 2	3 $\pm$ 1	3 $\pm$ 1	2 $\pm$ 1

Table 3. Subcellular distribution of radioactivity in rat liver and kidney 5 min after the injection of ^{125}I -labelled bovine somatotropin into female or male rats

Symbols (—) and (+) indicate the absence or the presence of excess of unlabelled bovine somatotropin in the injected solution. Values are means $\pm$ S.D. for determinations performed on three animals.

Fraction	Relative distribution of radioactivity (%)							
	Female				Male			
	Liver		Kidney		Liver		Kidney	
	—	+	—	+	—	+	—	+
Nuclear	18 $\pm$ 6	19 $\pm$ 6	28 $\pm$ 6	38 $\pm$ 1	18 $\pm$ 12	14 $\pm$ 4	25 $\pm$ 10	19 $\pm$ 8
Mitochondrial	10 $\pm$ 3	10 $\pm$ 3	32 $\pm$ 6	29 $\pm$ 0	8 $\pm$ 3	11 $\pm$ 2	31 $\pm$ 5	25 $\pm$ 3
Microsomal	58 $\pm$ 5	43 $\pm$ 9	22 $\pm$ 4	18 $\pm$ 3	56 $\pm$ 6	38 $\pm$ 5	23 $\pm$ 4	30 $\pm$ 0
100 000 g supernatant	14 $\pm$ 3	28 $\pm$ 2	18 $\pm$ 2	15 $\pm$ 2	18 $\pm$ 4	37 $\pm$ 4	21 $\pm$ 3	26 $\pm$ 6
Solubilized microsomes	47 $\pm$ 7	34 $\pm$ 7	9 $\pm$ 1	6 $\pm$ 0	40 $\pm$ 5	28 $\pm$ 6	6 $\pm$ 1	14 $\pm$ 4
Insoluble microsomes	9 $\pm$ 3	14 $\pm$ 5	9 $\pm$ 5	10 $\pm$ 3	13 $\pm$ 1	12 $\pm$ 2	10 $\pm$ 5	17 $\pm$ 4

*Gel-filtration analysis of the radioactivity solubilized from microsomal fractions labelled *in vivo* with ^{125}I -labelled human somatotropin*

In order to examine the nature of the radioactive material present in the microsomal fractions, they were solubilized with 1% (w/v) Triton X-100 and chromatographed on Sepharose CL-6B.

When solubilized liver microsomes from female rats injected with ^{125}I -labelled human somatotropin were subjected to chromatography (Fig. 1a), a major symmetrical peak of radioactivity was observed with K_D 0.31 ± 0.02 ($n = 8$) (all the distribution coefficients are expressed as means $\pm$ S.D. of the values obtained in n experiments). The small peak at the void volume and the peak detected at K_D 0.59 ± 0.01 ($n = 8$) correspond to aggregation products and to free ^{125}I -labelled human somatotropin

respectively. When ^{125}I -labelled human somatotropin was injected along with a 2700–4000-fold molar excess of unlabelled human somatotropin, nearly all the radioactivity was found to be free hormone (K_D 0.59 ± 0.01 , $n = 3$; Fig. 1b). Hence the formation of the large-molecular-size species is a saturable process.

Most of the radioactivity present in the detergent-solubilized microsomes from female kidney was free ^{125}I -labelled human somatotropin (K_D 0.60 ± 0.00 , $n = 5$; Fig. 1c). Small peaks at K_D 0.36 ± 0.02 ($n = 5$) and K_D 0.96 ± 0.02 ($n = 5$) were also observed. The first of these two peaks disappeared when there was a simultaneous injection of unlabelled human somatotropin (Fig. 1d) and presumably corresponds to a hormone-binding-protein complex. The other corresponds to degradation products.

Fig. 1. Sepharose CL-6B chromatography of the solubilized microsomal fractions from female rat liver (a and b) and kidney (c and d) obtained after the injection of ^{125}I -labelled human somatotropin alone (a and c) or with an excess of unlabelled human somatotropin (b and d)

Arrows indicate the position of the void volume and of the void plus the inner volume of the column respectively. The number adjacent to each peak denotes the mean K_D value calculated from the number of determinations cited in Table 4.

The elution profile corresponding to solubilized liver microsomes derived from male rats injected with ^{125}I -labelled human somatotropin showed three peaks (Fig. 2a). The first small peak (K_D 0) represents aggregation products; the second (K_D 0.24 ± 0.01 , $n = 11$) corresponds to a hormone-binding-protein complex, since it is abolished when unlabelled human somatotropin is administered together with the labelled hormone (Fig. 2b); the third peak (K_D 0.59 ± 0.01 , $n = 11$) represents free ^{125}I -labelled human somatotropin.

The formation of a ^{125}I -labelled human somatotropin-binding-protein complex was more evident in male than in female rat kidney (Fig. 2c). The K_D of this complex was 0.33 ± 0.01 ($n = 6$). Saturability of ^{125}I -labelled human somatotropin binding to the renal proteins is shown in Fig. 2(d).

In addition, the 100 000 g supernatants obtained under the different experimental conditions assayed were chromatographed on the same column. ^{125}I -

labelled human somatotropin was the predominant molecular species observed in all these cases. About 10% of the radioactivity from female liver supernatant was eluted at K_D 0.32, and 15% of the radioactive material in kidney supernatants corresponded to degradation products (results not shown).

Gel-filtration analysis of the radioactivity solubilized from microsomes labelled in vivo with ^{125}I -labelled bovine somatotropin

Gel-filtration chromatography of detergent-solubilized liver microsomes from female rats injected with ^{125}I -labelled bovine somatotropin showed a more complex pattern (Fig. 3a). Besides a small peak eluted at the void volume and a peak corresponding to free labelled hormone (K_D 0.59 ± 0.01 , $n = 3$), two more peaks were detected at K_D 0.23 ± 0.01 ($n = 3$) and 0.41 ± 0.01 ($n = 3$) respectively. Injection of unlabelled bovine somato-

Fig. 2. Sepharose CL-6B chromatography of the solubilized microsomal fractions from male rat liver (a and b) and kidney (c and d) obtained after the injection of ^{125}I -labelled human somatotropin alone (a and c) or with an excess of unlabelled human somatotropin (b and d)

Indications are otherwise identical with those described for Fig. 1.

Fig. 3. Sephadex CL-6B chromatography of the solubilized microsomal fractions from female rat liver (a and b) and kidney (c and d) obtained after the injection of ^{125}I -labelled bovine somatotropin alone (a and c) or with an excess of unlabelled bovine somatotropin (b and d)

Indications are otherwise identical with those described for Fig. 1.

tropin together with ^{125}I -labelled bovine somatotropin prevented the appearance of the first of these two peaks (Fig. 3b). This therefore represents a hormone-binding-protein complex. The second peak did not disappear when the excess of bovine somatotropin was administered. Analysis of the ^{125}I -labelled bovine somatotropin used to inject the animals revealed the presence of only free hormone (K_D 0.59), giving no evidence of the pre-existence of such molecular species. Hence the second peak was probably derived from a non-specific interaction of the labelled hormone with some component of the microsomal fraction. This would not be surprising, in view of the tendency of bovine somatotropin to form aggregates or to polymerize (Dellacha *et al.*, 1968; Fernández & Delfino, 1983).

The elution diagram corresponding to solubilized kidney microsomes from female rats injected with ^{125}I -labelled bovine somatotropin (Fig. 3c) displayed the non-specific peak (K_D 0.43 ± 0.00 , $n = 3$) and peaks of free labelled hormone (K_D 0.60 ± 0.02 ,

$n = 3$) and degradation products (K_D 0.88 ± 0.07 , $n = 3$). The simultaneous administration of unlabelled bovine somatotropin did not basically alter the characteristics of the diagram (Fig. 3d).

The injection of ^{125}I -labelled bovine somatotropin into male rats revealed a similar situation to that already described for female rats in both organs (Fig. 4 and Table 4).

The 100 000 g supernatants obtained in all the experiments performed with ^{125}I -labelled bovine somatotropin contained the non-specific species and free hormone. In addition, degradation products of the hormone were found in kidney supernatants (results not shown).

Analysis of gel-filtration parameters of hormone-binding-protein complexes formed in vivo

The Stokes radii of the hormone-binding-protein complexes were calculated from the relationship between K_D and the Stokes radii of several standard proteins. Table 4 summarizes the K_D value and the

Fig. 4. Sepharose CL-6B chromatography of the solubilized microsomal fractions from male rat liver (a and b) and kidney (c and d) obtained after the injection of ^{125}I -labelled bovine somatotropin alone (a and c) or with an excess of unlabelled bovine somatotropin (b and d)

The meaning of the arrows is the same as given in the legend to Fig. 1. This experiment was performed twice. The number adjacent to each peak denotes the K_D value obtained in an individual experiment.

Table 4. Distribution coefficients (K_D) and Stokes radii of hormone-binding-site complexes formed *in vivo* in rat liver and kidney

Wherever more than two determinations were made, results are expressed as means $\pm$ S.D., for the number of independent experiments (n) cited. The statistical significance of the differences indicated by ^a and ^b (Student's *t* test) is $P < 0.001$.

Organ	Hormone	Sex	K_D	Stokes radius (nm)	<i>n</i>
Liver	^{125}I -labelled human somatotropin	Female	0.31 ± 0.02^a	6.5 ± 0.4	8
		Male	$0.24 \pm 0.01^{a,b}$	7.9 ± 0.2	11
	^{125}I -labelled bovine somatotropin	Female	0.23 ± 0.01	8.1 ± 0.3	3
		Male	0.24, 0.23	7.9, 8.1	2
Kidney	^{125}I -labelled human somatotropin	Female	0.36 ± 0.02	5.6 ± 0.3	5
		Male	0.33 ± 0.01^b	6.1 ± 0.2	6

Stokes radii corresponding to these complexes. After taking into account the statistical significance of the differences between homologous parameters, data may be gathered into two main groups. One of them includes the complexes formed by ^{125}I -labelled human somatotropin in female liver and in male kidney, possessing Stokes radii of 6.1–6.5 nm. The

other includes the complexes formed by ^{125}I -labelled human somatotropin in male liver and by ^{125}I -labelled bovine somatotropin in female and male liver, possessing Stokes radii of 7.9–8.1 nm.

The value for the Stokes radius of the ^{125}I -labelled human somatotropin-binding-protein complex formed in female kidney may not be so reliable as the

others, because the small amount of complex prevented us from determining the K_D value accurately.

*Gel-filtration characteristics of the ^{125}I -labelled human somatotropin-binding-site complexes from rat liver formed *in vitro**

The elution pattern of solubilized liver microsomes from female rat pre-labelled *in vitro* with ^{125}I -labelled human somatotropin (Fig. 5a) was similar to that obtained *in vivo* (Fig. 1a). The solubilized labelled complex formed *in vitro* gave K_D 0.32 ± 0.01 ($n = 7$). This value is not significantly different from that obtained by labelling *in vivo* (Table 4). The K_D value of the complex observed after analysing solubilized liver microsomes from male rat pre-labelled *in vitro* with ^{125}I -labelled human somatotropin (Fig. 5c) was 0.35 ± 0.03 ($n = 4$). This value is significantly different ($P < 0.001$, Student's t test) from that obtained after labelling *in vivo* (Table 4).

Incubation with an excess of unlabelled human somatotropin inhibited the formation of the complex in both sexes (Figs. 5b and 5d).

Similar K_D values were obtained when the hormone-binding-site complexes were formed by incubating ^{125}I -labelled human somatotropin with solubilized extracts from female or male liver microsomes (Table 5).

Table 5. Distribution coefficients (K_D) and Stokes radii of ^{125}I -labelled human somatotropin-binding-site complexes formed *in vitro* in rat liver

Results are expressed as means $\pm$ s.d. for the numbers of determinations (n) cited.

Source	Sex	K_D	Stokes radius (nm)	n
Pre-labelled microsomes	Female	0.32 ± 0.01	6.3 ± 0.2	7
	Male	0.35 ± 0.03	5.8 ± 0.5	4
Solubilized extract	Female	0.32 ± 0.00	6.3 ± 0.0	5
	Male	0.33 ± 0.01	6.1 ± 0.2	3

Fig. 5. Sepharose CL-6B chromatography of the solubilized microsomal fractions from female (a and b) or male (c and d) rat liver pre-labelled *in vitro* with ^{125}I -labelled human somatotropin alone (a and c) or with an excess of unlabelled human somatotropin (b and d)

The meaning of the arrows is the same as given in the legend to Fig. 1. The number adjacent to each peak denotes the mean K_D value calculated from the numbers of determinations cited in Table 4.

Discussion

The two organs where a rapid concentration of human and bovine somatotropins occurs are clear examples of different ways of taking up hormones from the blood. Whereas the process is saturable in the liver, it is not saturable in the kidney. This statement is based on the quantitatively predominant process, and a small contribution of the other cannot be ruled out *a priori*. Our observations on this matter, although performed under moderately different conditions, basically confirm the previous findings reported by Turyn & Dellacha (1978).

In order to investigate further the characteristics of the uptake processes, the liver and the kidneys were homogenized and subjected to subcellular fractionation, a short time after injection of a radiolabelled somatotropin. Counting of radioactivity revealed that the greatest proportion of the hormone was specifically localized in the microsomal fraction of the liver. In contrast, the highest amount of radioiodinated hormone in the kidney was found in the 100 000 g supernatant, independently of the administration or not of excess of unlabelled hormone.

The uptake processes of both human somatotropin, which possesses growth (Li & Papkoff, 1956) as well as lactogenic activities (Chadwick *et al.*, 1961), and bovine somatotropin, which possesses only growth activity (Li *et al.*, 1945), seem to share common characteristics, since the distribution patterns in liver and kidney are very similar.

The results of our studies on the subcellular distribution of radiolabelled human or bovine somatotropins in liver differ considerably from those reported previously. Maddaiah *et al.* (1970) observed that ^3H -labelled human somatotropin injected into hypophysectomized rats was mainly concentrated in the mitochondrial and microsomal fractions. Rezvani *et al.* (1973) found that ^3H -labelled human somatotropin incubated with human liver slices was preferentially incorporated into a nuclear fraction. In contrast with the preceding observations, Groves *et al.* (1972) showed that most of the ^{125}I -labelled bovine somatotropin injected into immature female rats was localized in a cytosolic fraction of the liver. The mitochondrial was the particulate fraction that displayed the greatest amount of radioiodinated hormone bound. No definite conclusions may be drawn from comparison of these observations.

Apart from the different experimental conditions employed (hormones, animals, fractionation procedures etc.), we consider that the main factor involved in the generation of these discrepancies was the low specific radioactivity of the labelled hormones used as tracers. In fact, in no case did it reach

$1\mu\text{Ci}/\mu\text{g}$. As a consequence, their distribution was more probably related to non-specific phenomena. Because of the high affinity and the low quantity of binding sites in tissues, an analysis that comprises their influence in determining a particular subcellular distribution, with the aim of identifying a specific site of concentration, requires the employment of hormones with high specific radioactivity. This condition was fulfilled in our work by using hormones labelled up to about $100\mu\text{Ci}/\mu\text{g}$.

The concentration of human or bovine somatotropins *in vivo* by a saturable process in liver microsomal fraction might have arisen from the existence of specific binding sites in some of its components. Sites for lactogenic (Posner *et al.*, 1974a,b; Herington *et al.*, 1976a,b; Postel-Vinay, 1976; Ranke *et al.*, 1976a,b; Postel-Vinay & Desbuquois, 1977; Dang Vu & Bénard, 1978; Bonifacino *et al.*, 1978, 1981; Silverstein & Richards, 1979; Mountjoy *et al.*, 1980) and growth hormones (Eitzrodt *et al.*, 1976; Ranke *et al.*, 1976a,b; Postel-Vinay, 1976; Turyn *et al.*, 1976; Postel-Vinay & Desbuquois, 1977; Baxter *et al.*, 1980) have been described in rat liver. Coincidentally, they were found mainly in the microsomal fraction. As demonstrated by Bergeron *et al.* (1978) and confirmed by Finidori *et al.* (1980), binding sites for ^{125}I -labelled human somatotropin are localized in Golgi and plasmalemma fractions.

However, the existence of other kinds of saturable processes, such as transport of the free radioiodinated hormones into membrane vesicles, could not be discarded. With the purpose of verifying the participation of binding sites in the uptake of somatotropins *in vivo*, the microsomal fractions were solubilized and subjected to gel-filtration analysis on Sepharose CL-6B.

By using this approach, it was demonstrated that ^{125}I -labelled human somatotropin forms specific complexes *in vivo*, with Stokes radii of 6.1–6.5 nm in female liver and in male kidney. Furthermore, specific complexes with Stokes radii of 7.9–8.1 nm are formed *in vivo* by ^{125}I -labelled human somatotropin in male liver and by ^{125}I -labelled bovine somatotropin in female and male liver. A hypothesis based on studies performed *in vitro* may be enunciated to explain this rather complicated picture. Complexes belonging to the first group are originated by interaction with binding proteins specific for lactogenic hormones ('lactogenic'), whereas those belonging to the second group are originated by interaction with binding proteins specific for hormones with growth activity ('somatogenic'). The following observations made *in vitro* provide supportive evidence for this hypothesis. (a) Characterization of ^{125}I -labelled human somatotropin binding to particulate (Posner *et al.*, 1974a,b; Posner, 1976; Herington *et al.*, 1976b; Moore &

Jin, 1978) or solubilized (Bonifacino *et al.*, 1978, 1981; Du Caju *et al.*, 1978) subcellular fractions from female rat liver demonstrated the lactogenic specificity of the interaction. Although somatogenic binding sites are also present in these preparations (see point *d*), their number (Baxter *et al.*, 1980) is very low in comparison with that of lactogenic binding sites (Bonifacino *et al.*, 1981). As a result, only the lactogenic sites and their characteristics may be detected by using ^{125}I -labelled human somatotropin as ligand. (*b*) The presence of binding sites specific for lactogenic hormones was described in rat (Marshall *et al.*, 1975; Mountjoy *et al.*, 1980) and rabbit kidney (Roguin *et al.*, 1981, 1982). Mountjoy *et al.* (1980) reported that the specific binding activity is about 5 times higher in male than in female rat kidney. Somatogenic binding sites were not detected in this organ. (*c*) Binding of ^{125}I -labelled human somatotropin to liver microsomes from male rats was shown to have somatogenic characteristics (Postel-Vinay, 1976). (*d*) The existence of somatogenic binding sites in liver microsomal fraction from female or male rats was demonstrated by using ^{125}I -labelled bovine somatotropin as ligand (Baxter *et al.*, 1980).

The ^{125}I -labelled human somatotropin-binding-protein complexes formed *in vivo* and *in vitro* in liver microsomes from female rats displayed virtually identical gel-filtration characteristics. This observation suggests that the studies on the properties of the lactogenic binding sites performed in artificial media are valid, since the same macromolecular complex is formed under physiological conditions. This approach was previously employed for demonstrating the biological relevance of lutropin/choriogonadotropin receptors (Dufau *et al.*, 1975; Bellisario & Bahl, 1975).

An important discrepancy arises from the comparison of the Stokes radii of the ^{125}I -labelled human somatotropin-binding-protein complexes formed in male rat liver *in vivo* ($7.9 \pm 0.2\text{ nm}$, mean $\pm$ S.D., $n = 11$) or *in vitro* ($5.8 \pm 0.5\text{ nm}$, mean $\pm$ S.D., $n = 4$). Since the fractionation and solubilization procedures and the gel-filtration analysis were performed under identical conditions, an artifact origin of this difference seems unlikely. Several phenomena may be proposed as causes of the discrepancy. Among them, one interesting possibility is that the complex formed *in vitro* is an intermediate step in the genesis of the complex formed *in vivo*. However, much more experimental work is needed to clarify this question.

Analysis of the radioactive molecules present in the solubilized microsomes provided a conclusive view on the uptake processes of somatotropins into liver and kidney. The liver uptake is mainly due to binding of the hormones to specific sites. This explains the saturability and all the other charac-

teristics (Turyn & Dellacha, 1978) of this process. These observations are in agreement with those made by Josefsberg *et al.* (1979), who demonstrated that the uptake of ^{125}I -labelled sheep prolactin in female rat liver is mediated by receptors, with particular concentration in the Golgi elements of the cell. In contrast, binding to specific sites represents only a small proportion of the uptake of lactogenic hormones by the kidney. The main process in this organ is fundamentally non-specific. Such hormones, like many other low-molecular-weight proteins, are concentrated into the kidney by glomerular filtration followed by tubular resorption. The small contribution of the specific process and a higher individual variability are the reasons why a decrease in the kidney/blood ratio was not observed when the injected labelled hormone was accompanied with excess of unlabelled hormone.

We thank Dr. J. M. Dellacha for helpful discussions and criticism. This work was supported in part by grants from the Universidad de Buenos Aires, the Consejo Nacional de Investigaciones Científicas y Técnicas and the Regional Program of Scientific and Technical Development of the O.A.S.

References

- Baxter, R. C., Bryson, J. M. & Turtle, J. R. (1980) *Endocrinology* **107**, 1176–1181
- Bellisario, R. & Bahl, O. P. (1975) *J. Biol. Chem.* **250**, 3837–3844
- Bergeron, J. J. M., Posner, B. I., Josefsberg, Z. & Sikstrom, R. (1978) *J. Biol. Chem.* **253**, 4058–4066
- Birkinshaw, M. & Falconer, I. R. (1972) *J. Endocrinol.* **55**, 323–334
- Bonifacino, J. S., Sánchez, S. H. & Paladini, A. C. (1978) *Biochem. Biophys. Res. Commun.* **85**, 62–69
- Bonifacino, J. S., Sánchez, S. H. & Paladini, A. C. (1981) *Biochem. J.* **194**, 385–394
- Chadwick, A., Folley, S. J. & Gemzell, C. A. (1961) *Lancet* **ii**, 241–243
- Dang Vu, T. K. & Bénard, B. (1978) *Ann. Endocrinol.* **39**, 451–462
- Dellacha, J. M., Enero, M. A. & Paladini, A. C. (1968) *Biochim. Biophys. Acta* **168**, 95–105
- Du Caju, M., Postel-Vinay, M. C. & Desbuquois, B. (1978) *Arch. Fr. Pédiatr.* **35**, Suppl., 102–114
- Dufau, M. L., Podestà, E. J. & Catt, K. J. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1272–1275
- Durán-García, S., Obregón, M. J., Morreale de Escobar, G. & Escobar del Rey, F. (1981) *Endocrinology* **108**, 2054–2059
- Etzrodt, H., Musch, K. A., Schleyer, M. & Pfeiffer, E. F. (1976) *J. Clin. Endocrinol. Metab.* **42**, 1184–1187
- Fernández, H. N. & Delfino, J. M. (1983) *Biochem. J.* **209**, 107–115
- Finidori, J., Postel-Vinay, M. C. & Kleinknecht, C. (1980) *Endocrinology* **106**, 1960–1965
- Groves, W. E., Houts, G. E. & Bayse, G. S. (1972) *Biochim. Biophys. Acta* **264**, 472–480
- Herington, A. C., Phillips, L. S. & Daughaday, W. H. (1976a) *Metab. Clin. Exp.* **25**, 341–353

- Herington, A. C., Veith, N. H. & Burger, H. G. (1976b) *Biochem. J.* **158**, 61–69
- Johnson, V. & Maack, T. (1977) *Am. J. Physiol.* **233**, F185–F196
- Josefsberg, Z., Posner, B. I., Patel, B. & Bergeron, J. J. M. (1979) *J. Biol. Chem.* **254**, 209–214
- Li, C. H. & Papkoff, H. (1956) *Science* **124**, 1293–1294
- Li, C. H., Evans, H. M. & Simpson, M. E. (1945) *J. Biol. Chem.* **159**, 353–366
- Maddaiah, V. T., Rezvani, I., Chen, S. Y. & Collipp, P. J. (1970) *Biochem. Med.* **4**, 494–499
- Marshall, S., Gelato, M. & Meites, J. (1975) *Proc. Soc. Exp. Biol. Med.* **149**, 185–188
- Moore, W. V. & Jin, D. (1978) *J. Clin. Endocrinol. Metab.* **46**, 374–380
- Mountjoy, K., Cowden, E. A., Dobbie, J. W. & Ratcliffe, J. G. (1980) *J. Endocrinol.* **87**, 47–54
- Posner, B. I. (1976) *Endocrinology* **99**, 1168–1177
- Posner, B. I., Kelly, P. A. & Friesen, H. G. (1974a) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2407–2410
- Posner, B. I., Kelly, P. A., Shiu, R. P. C. & Friesen, H. G. (1974b) *Endocrinology* **95**, 521–531
- Postel-Vinay, M. C. (1976) *FEBS Lett.* **69**, 137–140
- Postel-Vinay, M. C. & Desbuquois, B. (1977) *Endocrinology* **100**, 209–215
- Ranke, M. B., Stanley, C. A., Rodbard, D., Baker, L., Bongiovanni, A. M. & Parks, J. S. (1976a) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 847–851
- Ranke, M. B., Stanley, C. A., Tenore, A., Rodbard, D., Bongiovanni, A. M. & Parks, J. S. (1976b) *Endocrinology* **99**, 1033–1045
- Retegui-Sardou, L. A., Scaramal, L. O., Dellacha, J. M. & Paladini, A. C. (1977) *Mol. Cell. Biochem.* **16**, 87–96
- Rezvani, I., Maddaiah, V. T., Collipp, P. J., Thomas, J. & Chen, S. Y. (1973) *Biochem. Med.* **7**, 432–440
- Roguin, L. P., Sánchez, S. H., Bonifacino, J. S. & Paladini, A. C. (1981) *Biochem. J.* **200**, 257–264
- Roguin, L. P., Bonifacino, J. S. & Paladini, A. C. (1982) *Biochim. Biophys. Acta* **715**, 222–229
- Silverstein, A. M. & Richards, J. F. (1979) *Biochem. J.* **178**, 743–751
- Turyn, D. & Dellacha, J. M. (1978) *Endocrinology* **103**, 1190–1195
- Turyn, D., Santomé, J. A. & Dellacha, J. M. (1976) *Acta Physiol. Latinoam.* **26**, 395–402