Human somatotropin binding to rabbit kidney microsomal fraction

Leonor P. ROGUIN, Susana H. SÁNCHEZ, Juan S. BONIFACINO and Alejandro C. PALADINI
*Departamento, de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.
Junín 956, 1113 Buenos Aires, Argentina*

(Received 8 May 1981/Accepted 14 July 1981)

Specific binding of ^{125}I -labelled human somatotropin was demonstrated in microsomal membranes (microsomes) from rat and rabbit kidneys. Female rabbit kidney microsomes showed the highest binding activity and were used for further study. The association of ^{125}I -labelled human somatotropin was time- and temperature-dependent and the binding reaction was reversible. Scatchard analysis of saturation data indicated a dissociation equilibrium constant, K_D , of 56 pM and a binding capacity of 37 fmol per mg of protein. Similar results were obtained from competition experiments. Binding of ^{125}I -labelled human somatotropin to the microsomes was specifically inhibited by hormones with lactogenic activity. The binding sites, as well as ^{125}I -labelled human somatotropin, were not inactivated on incubation. Treatment of the microsomes with trypsin and chymotrypsin decreased the specific binding by over 90%. Preheating of the microsomes at 55°C for 15 min abolished 50% of the specific binding activity.

Distribution studies of radioactively labelled somatotropins injected into animals from different species revealed that the kidney was the organ where the highest concentration of radioactivity occurred (Sonenberg *et al.*, 1954; Salmon *et al.*, 1962; Collipp *et al.*, 1966; Wallace *et al.*, 1972; Retegui-Sardou *et al.*, 1977; Johnson & Maack, 1977).

On the basis of these studies it has been suggested that somatotropin is removed from blood by glomerular filtration and then subjected to tubular reabsorption with subsequent passage of the intact hormone or its metabolic products back into the blood. A simultaneous effect of the hormone on kidney function may occur during this process (Wallace *et al.*, 1972; Retegui-Sardou *et al.*, 1977).

Autoradiographic studies demonstrated the localization of somatotropins in the convoluted tubules of the kidney (Mess & Hámori, 1965; De Kretser *et al.*, 1969; Collipp *et al.*, 1966; Stacy *et al.*, 1976).

Prolactin, which is structurally and functionally related to somatotropin, was also concentrated by the kidney after intravenous injection (Birkinshaw & Falconer, 1972) with localization either in the proximal tubules, as was shown by autoradiographic (Rajaniemi *et al.*, 1974) and double-antibody (Donatsch & Richardson, 1975) techniques or in the loop of Henle, distal tubule and collecting duct (Evan *et al.*, 1977).

These findings are consistent with the possible presence of specific binding sites in kidney. In order

to verify this hypothesis the binding of human somatotropin, which possesses growth (Li & Papkoff, 1956) as well as lactogenic (Kleinberg & Frantz, 1971) activity, was investigated in rat and rabbit kidney and was characterized in female rabbit kidney microsomes.

Materials and methods

Materials

The majority of the hormones and chemicals used in this work were prepared, purified or obtained as indicated by Bonifacino *et al.* (1981).

Follitropin and lutropin were obtained from N.I.H., N.I.A.M.D.D. Hormone Distribution Program, Bethesda, MD, U.S.A. and chymotrypsin from bovine pancreas was from Sigma.

Iodination of human somatotropin

^{125}I -labelled somatotropin was prepared as described previously (Bonifacino *et al.*, 1981).

Crude kidney microsomal preparation

Kidneys were obtained from adult male or adult virgin female New Zealand rabbits (3.0–3.5 kg), and from adult male or adult virgin female rats of the Long–Evans strain (210–250 g).

The animals were killed by cervical dislocation and the kidneys quickly removed and processed, or kept at –60°C for less than 1 month.

The fresh, or thawed, kidneys were rinsed with a

0.3 M-sucrose/0.5 mM-CaCl₂/5 mM-Tris/HCl buffer, pH 7.0, and homogenized for 90 s at 0°C in 6 vol. (v/w) of the same buffer, in a Sorvall Omni-Mixer at a speed setting of 5.

The homogenate was centrifuged for 20 min at 15000 g (r_{av} . 7.4 cm) at 4°C in a Sorvall RC2-B centrifuge with an SS34 rotor. The supernatant was centrifuged for 90 min at 100000 g (r_{av} . 5.9 cm) at 4°C in a type 40 rotor of a Spinco model L ultracentrifuge and the pellet was suspended in 25 mM-Tris/HCl buffer, pH 7.0, with a Dounce-type homogenizer. A sample of the suspension was solubilized by heating for 30 min at 100°C in 1 M-NaOH and the protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

The crude microsomal suspension, fractionated into 1–2 ml portions, was kept at –60°C until used.

Measurement of ¹²⁵I-labelled human somatotropin binding

Binding assays were performed by incubating, for 16 h at 25°C, 1.6–2.2 ng of ¹²⁵I-labelled human somatotropin (80000–100000 c.p.m.) with 400–600 µg of microsomal proteins in a total volume of 0.5 ml. Plastic tubes were used and the incubation medium consisted of 25 mM-Tris/HCl (pH 7.0)/10 mM-CaCl₂/0.08% bovine serum albumin. In each determination, tubes containing the same mixture described plus 5 µg of unlabelled human somatotropin were incubated in parallel.

The binding was terminated by dilution with 4 ml of cold incubation medium and centrifugation at 1400 g (r_{av} . 15 cm) for 25 min at 4°C. The supernatant was discarded and the tubes were kept inverted on filter paper for at least 12 h at 4°C before being counted for radioactivity.

All determinations were carried out in triplicate and the results are expressed as mean values. The standard deviation was always less than 3%. The percentage of specific binding was calculated as $(C_1 - C_2) \times 100/A$ where C_1 is the amount of radioactivity bound in the absence of unlabelled human somatotropin, C_2 is the amount of radioactivity bound in the presence of 5 µg of unlabelled human somatotropin and A is the total amount of radioactivity initially placed in each tube.

Results

Specific binding of ¹²⁵I-labelled human somatotropin to rat and rabbit kidney microsomes

Table 1 shows the binding activity of ¹²⁵I-labelled human somatotropin to kidney microsomes from adult rats or rabbits.

The specific binding to the microsomes preparations varies with the sex of the animals used; the binding was higher in female rabbit membranes

Table 1. Specific binding of ¹²⁵I-labelled human somatotropin to rat and rabbit kidney microsomes

¹²⁵I-labelled human somatotropin (100 fmol) was incubated with 500–600 µg of microsomal proteins and binding was measured as described in the Materials and methods section. Specific activity is the amount (fmol) of labelled hormone specifically bound per mg of protein. The numbers in parentheses indicate the number of animals used to prepare the membranes. Results are means $\pm$ s.d. of the number of experiments in parentheses.

Animal species	Sex	Specific activity (fmol/mg)
Rat	Female	9.5 $\pm$ 0.6 (4)
Rat	Male	25.1 $\pm$ 0.8 (4)
Rabbit	Female	32.8 $\pm$ 0.4 (3)
Rabbit	Male	18.8 $\pm$ 1.3 (3)

Table 2. Specific binding of ¹²⁵I-labelled human somatotropin to different female rabbit kidney fractions

The kidneys were processed and analysed as indicated in the Materials and methods section. The binding of ¹²⁵I-labelled human somatotropin to the 100000 g supernatant was measured by the poly(ethylene glycol) precipitation method (Bonifacino *et al.*, 1981). The specific activity is expressed as indicated in Table 1. Results are means $\pm$ s.d. obtained from three independent experiments.

Subcellular fraction	Specific activity (fmol/mg)
Homogenate	10.4 $\pm$ 2.1
15000 g pellet	12.5 $\pm$ 0.1
100000 g pellet	19.2 $\pm$ 0.7
100000 g supernatant	0.7 $\pm$ 0.3

than in male ones, whereas the inverse situation was observed in rats. Microsomes from female rabbits were selected for further study.

Distribution of ¹²⁵I-labelled human somatotropin binding activity in subcellular fractions of female rabbit kidney.

The binding activity of ¹²⁵I-labelled human somatotropin to subcellular fractions obtained by homogenization and differential centrifugation of renal tissue is shown in Table 2. The crude microsomal suspension obtained with the 100000 g pellet showed the highest specific activity and was used in this study.

Effect of membrane protein concentration on the specific binding of ¹²⁵I-labelled human somatotropin

The specific binding increases in direct proportion to the membrane protein concentration (Fig. 1) up to a value of 800 µg per tube. Accordingly, all binding experiments were performed with 400–600 µg of protein per tube.

Fig. 1. Effect of membrane protein concentration on the specific binding of ¹²⁵I-labelled human somatotropin. ¹²⁵I-labelled human somatotropin (100000 c.p.m.) was incubated for 16 h at 25°C with increasing amounts of rabbit kidney membrane proteins. The specific (●) and non-specific (○) binding were determined as indicated in the Materials and methods section.

Fig. 2. Effect of time and temperature on the binding of ¹²⁵I-labelled human somatotropin to rabbit kidney membranes.

¹²⁵I-labelled human somatotropin (100 fmol) was incubated for various times with 500 μg of microsomal proteins at the indicated temperatures. The specific binding was measured at 37°C (○), 25°C (●), 15°C (□) and 4°C (■).

Kinetics of association

The specific binding of ¹²⁵I-labelled human somatotropin to kidney microsomes is time- and temperature-dependent, as shown in Fig. 2. At 25°C maximal binding is obtained within 15 h and this value remains constant for at least 30 h. At 15°C, or at 4°C, equilibrium is not reached in 30 h, the longest incubation time explored. At 37°C the association progresses faster than at 25°C and reaches essentially the same equilibrium level; the binding then decreases, probably due to inactivation or degradation of the binding sites, the hormone, or both.

Kinetic analysis of the values obtained at 25°C shows that the association reaction obeys second-order kinetics. The mean value of the association rate constant (k_{+1}) at 25°C, obtained from three similar experiments, was $(1.1 \pm 0.4) \times 10^7 \text{ mol}^{-1} \cdot \text{litre} \cdot \text{min}^{-1}$.

Kinetics of dissociation

The specific binding of ¹²⁵I-labelled human somatotropin to kidney microsomes is reversible: approx. 50% of specifically bound hormone is dissociated after 30 h at 25°C (Fig. 3). The rate of this process is unaffected by the presence of unlabelled hormone (2500-fold molar excess).

When the dissociation data are represented as the variation of the logarithm of the concentration of hormone-binding site complex with time, a linear

Fig. 3. Dissociation of ¹²⁵I-labelled human somatotropin from the hormone binding site complex at 25°C.

¹²⁵I-labelled human somatotropin (80000 c.p.m.) was incubated with 500 μg of microsomal protein under the conditions described in the Materials and methods section. The pellets obtained were suspended in 4.0 ml of incubation medium (●) or 4.0 ml of incubation medium plus 5 μg of unlabelled human somatotropin (○), and then were incubated at 25°C for a variety of time intervals, before centrifuging at 1400 g for 25 min at 4°C. The new pellets were counted for radioactivity.

Fig. 4. Specific binding of ^{125}I -labelled human somatotropin as a function of labelled hormone concentration (a) A constant amount of microsomal proteins ($550\text{ }\mu\text{g}$) was incubated with increasing amounts of ^{125}I -labelled human somatotropin for 16 h at 25°C . The specific (●) and non-specific (○) binding were measured as indicated in the Materials and methods section. (b) Scatchard analysis of the binding data from the experiment shown in (a).

relationship is obtained, indicating first-order kinetics. The mean value of the dissociation rate constant (k_{-1}) obtained from two similar experiments was $3.8 \times 10^{-4} \text{ min}^{-1}$.

Effect of hormone concentration on the specific binding

Fig. 4(a) shows that the specific binding of ^{125}I -labelled human somatotropin to the microsomes is a saturable process, whereas the non-specific binding increases linearly.

Scatchard (1949) analysis of the binding data from Fig. 4(a) yields a linear plot (Fig. 4b). The specific binding sites in the microsomes have $K_D = 56 \pm 18 \text{ pM}$ and a binding capacity of $37 \pm 5 \text{ fmol/mg}$ of protein (means $\pm$ s.d.; $n = 5$).

Specificity of ^{125}I -labelled human somatotropin binding to rabbit kidney microsomes

Fig. 5 shows the inhibition curves obtained when the specific binding of ^{125}I -labelled human somatotropin to the microsomes is challenged by different hormones. Lactogenic hormones, such as ovine prolactin and human choriomammotropin, inhibit the binding of ^{125}I -labelled human somatotropin, although less effectively than does human somatotropin.

The effect observed with bovine somatotropin was minimal, since only a 30% inhibition was observed with a concentration three orders of magnitude higher than an equi-effective dose of human somatotropin. Equine somatotropin and other un-

Fig. 5. Competition curves for the specific binding of ^{125}I -labelled human somatotropin to rabbit kidney microsomes

^{125}I -labelled human somatotropin ($80\,000 \text{ c.p.m.}$) was incubated with $450\text{ }\mu\text{g}$ of microsomal protein in the presence of increasing amounts of human somatotropin (●), ovine prolactin (○), human choriomammotropin (Δ) and bovine somatotropin ($\blacktriangle$). Only a single dose of follitropin ($\blacksquare$), insulin, lutropin and equine somatotropin (all $\square$) was assayed. The procedure was described in the Materials and methods section. Results are expressed as the ratio of the specific binding at each concentration of unlabelled hormone (B) to the specific binding in the absence of additions (B_0).

related hormones (follitropin, lutropin and insulin), do not inhibit the specific binding.

Scatchard plots obtained from competition data were also linear. The values of the dissociation equilibrium constant and binding capacity calculated by this procedure were 48 ± 11 pM and 40 ± 3 fmol/mg respectively. These parameters are similar to those obtained from saturation curves.

When kidney microsomes from adult male rabbits were assayed, the resulting inhibition curves indicated a pattern of specificity similar to that obtained with female animals.

Binding site and 125 I-labelled human somatotropin inactivation during incubation

In order to study the inactivation of the binding sites, membranes were pre-incubated for 16 h at 25°C under standard conditions. Then 125 I-labelled human somatotropin was added and the reaction mixture was incubated for 7 h at 25°C. No differences were found in comparison with a sample of microsomes that was not pre-incubated.

The possible degradation of the hormone was investigated by incubating 125 I-labelled human somatotropin (200 000 c.p.m.) with the microsomal suspension (600 µg of protein) for 16 h at 25°C in the usual medium. The integrity of the labelled hormone was analysed by chromatography on Sephadex G-100 (Bonifacino *et al.*, 1978) and by its ability to rebind to fresh microsomes.

The gel filtration experiments were done as follows. The bound hormone was dissociated by adjusting the incubation medium to pH 3.0 with 1 M-HCl. After 5 min at 25°C the reaction mixture was neutralized (pH 7.0) with 1 M-NaOH and the membranes were eliminated by centrifugation at 1400 g for 25 min at 4°C. More than 90% of the hormone in the complex was released by this treatment. The supernatant, which contained unbound as well as dissociated hormone, was submitted to chromatography on Sephadex G-100. No indications of degradation were found in the elution pattern.

The rebinding experiments were carried out in the following way. Reaction mixture that had been incubated for 16 h at 25°C was centrifuged at 1400 g for 25 min at 4°C. The supernatant (free labelled hormone) was kept for later studies. The pellet (bound labelled hormone) was washed with 25 mM-Tris / HCl (pH 7.0) / 10 mM-CaCl₂ / 0.08% bovine serum albumin and then was resuspended in the same medium adjusted to pH 3.0 with 1 M-HCl. After 5 min at 25°C the suspension was neutralized (pH 7.0) with 1 M-NaOH and the dissociated 125 I-labelled hormone was obtained by centrifugation.

Solutions of non-treated, unbound and bound labelled hormone (20 000 c.p.m.) were incubated

with fresh microsomal suspension (140 µg of protein) under standard conditions. The percentages of specific binding were 6.7 ± 0.3 , 6.4 ± 0.8 and 6.5 ± 0.7 (means $\pm$ s.d.; $n = 3$) respectively. The conclusion was drawn that on incubation with the microsomes the labelled hormone is neither degraded nor inactivated.

Effect of different enzymic treatments on the binding activity of the microsomal membranes

To explore the chemical nature of the binding sites, the microsomes were treated with different enzymes (Table 3). Trypsin and chymotrypsin caused more than a 90% decrease in the specific binding, whereas treatment with neuraminidase or phospholipase C had no effect. Phospholipase A₂ caused a 40% increase in the specific binding.

Sensitivity of the binding sites to heat treatment

Fig. 6 shows that the binding sites in the microsomes are unaffected by treatment for 15 min at 45°C. Any further increase in the temperature

Table 3. *Effect of different enzymic digestions on binding of 125 I-labelled human somatotropin to rabbit kidney microsomes*

The microsomal suspension (5 mg of protein) was incubated with the enzymes for 30 min at 37°C in 1 ml of the following media: trypsin and chymotrypsin, 25 mM-Tris/HCl buffer, pH 7.0; neuraminidase, 25 mM-Tris/HCl buffer, pH 5.0 in the presence of 0.1% bovine serum albumin; phospholipases C and A₂, 25 mM-Tris/HCl buffer, pH 7.0/2 mM-CaCl₂/0.1% bovine serum albumin. Trypsin action was stopped with ovomucoid trypsin inhibitor at a final concentration of 600 µg/ml. At the end of the incubation period each reaction mixture was diluted 30-fold with 25 mM-Tris/HCl buffer, pH 7.0/0.1% bovine serum albumin and centrifuged for 1 h at 100 000 g (r_{av} , 5.9 cm) at 4°C in a type 40 rotor of a Spinco L ultracentrifuge. The pellets were suspended in 1 ml of 25 mM-Tris/HCl buffer, pH 7.0. In each case a control without enzyme was carried out in parallel. The specific binding was measured as indicated in the Materials and methods section. Results are means $\pm$ s.d. from three independent experiments.

Enzyme	Enzyme/ microsomal protein ratio (w/v)	Specific binding of 125 I-labelled human somatotropin (% of control)
None (control)	—	100
Trypsin	1:30	9.2 ± 0.4
Chymotrypsin	1:30	7.6 ± 0.3
Neuraminidase	1:600	105.0 ± 5.6
Phospholipase C	1:30	95.0 ± 2.6
Phospholipase A ₂	1:150	140.4 ± 12.4

Fig. 6. *Sensitivity of the receptors to heat treatment* Microsomal suspension (20 mg of protein, 1 ml) was treated for 15 min at the temperature indicated. Specific binding was measured by incubating ^{125}I -labelled human somatotropin (100 000 c.p.m.) with 20 μl of the treated membrane preparation as indicated in the Materials and methods section. The results are expressed as percentages of binding of a homologous untreated preparation (control).

rapidly inactivates the specific binding activity; 50% remains at 55°C and 0% at 65°C.

These results, together with the reported inactivation of the binding sites by trypsin or chymotrypsin action, suggest the importance of a protein component in the human somatotropin binding sites.

Discussion

The present studies demonstrate the existence of specific binding sites for ^{125}I -labelled human somatotropin in microsomal membranes from rat and rabbit kidneys. The characteristics of the binding reaction were further investigated in female rabbit microsomes, which showed the highest binding activity.

The interaction displayed many of the properties required to define a hormone receptor, such as high affinity, structural specificity, saturability and reversibility (Cuatrecasas, 1974; Cuatrecasas & Hollenberg, 1976; Posner, 1975; Kahn, 1976; Catt & Dufau, 1976, 1977).

The kinetics of the association process is second order and analysis of the dissociation kinetics indicates that this process is first order. These results, together with the linear nature of the Scatchard plots whether saturation or competition

data are used, strongly suggest the homogeneity of the binding sites studied.

It is well known that ^{125}I -labelled human somatotropin can be bound by two types of binding sites, which, according to their specificity were called 'somatogenic' (Ranke *et al.*, 1976a,b) and 'lactogenic' (Posner *et al.*, 1974; Postel-Vinay, 1976).

Binding of ^{125}I -labelled human somatotropin to the somatogenic sites is specifically inhibited by hormones possessing growth activity, whereas hormones with lactogenic activity only compete for binding to the lactogenic sites (Tsushima & Friesen, 1973; Posner *et al.*, 1974; Kelly *et al.*, 1974; Herington *et al.*, 1976; Ranke *et al.*, 1976a,b; Postel-Vinay, 1976; Postel-Vinay & Desbuquois, 1977). The heterogeneity of both somatogenic (Turyn *et al.*, 1976; Posner, 1976; Hughes, 1979; Baxter *et al.*, 1980) and lactogenic (Herington *et al.*, 1976) sites has been postulated. Furthermore, some studies indicated an overlapping of their specificity. Thus, prolactin recognizes somatogenic binding sites (Ranke *et al.*, 1976a; Waters & Friesen, 1979; Cadman & Wallis, 1980; Wallis & Cadman, 1981) and bovine somatotropin can be bound by lactogenic sites (Ranke *et al.*, 1976a; Cadman & Wallis, 1980; Wallis & Cadman, 1981), although with a lower affinity than that of the corresponding hormones. These phenomena have impeded a clear distinction between the two classes of sites in some systems.

However, the specificity studies performed with our preparation showed the presence of binding sites which interact only with lactogenic hormones, such as human somatotropin, ovine prolactin and human choriomammotropin. The existence of a small proportion of somatogenic binding sites, undetectable under the present experimental conditions, cannot be ruled out.

It is noteworthy that the binding sites described in this paper possess one of the highest affinities reported for receptors with similar specificity (Kelly *et al.*, 1974; Shiu & Friesen, 1974; Frantz *et al.*, 1974; Aragona & Friesen, 1975; Sakai *et al.*, 1975; Herington *et al.*, 1976; Dang Vu & Bénard, 1978; Marshall *et al.*, 1978; Manni *et al.*, 1978; Silverstein & Richards, 1979; Mountjoy *et al.*, 1980; Bonifacino *et al.*, 1981).

Lactogenic sites were also identified in mouse (Frantz *et al.*, 1974) and rat (Marshall *et al.*, 1975; Mountjoy *et al.*, 1980) kidneys using ^{125}I -labelled ovine prolactin as a ligand. In addition the regulation of these binding sites by dehydration (Marshall *et al.*, 1975), oestrogens and testosterone (Marshall *et al.*, 1976), glucocorticoids (Marshall *et al.*, 1978) and thyroid status (Marshall *et al.*, 1979) was demonstrated.

Treatment of the membranes with phospholipase A_2 causes an increase in the total binding activity. This phenomenon is similar to that reported for the

insulin receptor in liver and fat cell membranes (Cuatrecasas, 1971) and for the human somatotropin binding sites in liver microsomes (Du Caju *et al.*, 1978; Sánchez *et al.*, 1979) or isolated hepatocytes (Postel-Vinay & Desbuquois, 1977). An increase in binding activity was also achieved by adding Triton X-100 to the membranes (Cuatrecasas, 1972; Bonifacino *et al.*, 1981). Although a change in affinity cannot be discarded, we favour the view of exposure of masked receptors, either inhibited by phospholipids or occluded into membrane vesicles.

At present, the role played by the lactogenic sites in the kidney is unknown. Since this organ is one of the major sites of degradation of somatotropin and prolactin (Retegui-Sardou *et al.*, 1977; Birkinshaw & Falconer, 1972), it is possible that the binding sites are involved in their catabolism. However, some evidence indicates that these hormones may be important in the regulation of water and electrolyte balance in various mammals, including man. It has been reported that they decrease the urinary excretion of water, sodium and potassium in humans (Biglieri *et al.*, 1961; Horrobin *et al.*, 1971), cats (Lockett & Roberts, 1963; Lockett, 1965) and rats (Lees *et al.*, 1964; Lockett & Nail, 1965; Wallin & Lee, 1976). Similar renal effects have been demonstrated only for prolactin in sheep (Horrobin *et al.*, 1973) and rabbits (Burstyn, 1976).

Thus, the lactogenic binding sites may represent physiological receptors, involved in the mechanism of regulation of renal function in mammals.

We thank Dr. J. M. Dellacha and other members of this Department 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

- Aragona, C. & Friesen, H. G. (1975) *Endocrinology* **97**, 677–684
- Baxter, R. C., Bryson, J. M. & Turtle, J. R. (1980) *Endocrinology* **107**, 1176–1181
- Biglieri, E. G., Watlington, C. O. & Forsham, P. H. (1961) *J. Clin. Endocrinol. Metab.* **21**, 361–370
- 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
- Burstyn, P. G. (1976) *J. Endocrinol.* **68**, 15p
- Cadman, H. F. & Wallis, M. (1980) *J. Endocrinol.* **87**, 53p–54p
- Catt, K. J. & Dufau, M. L. (1976) *Biol. Reprod.* **14**, 1–15
- Catt, K. J. & Dufau, M. L. (1977) *Annu. Rev. Physiol.* **39**, 529–557
- Collipp, P. J., Patrick, J. R., Goodheart, C. & Kaplan, S. A. (1966) *Proc. Soc. Exp. Biol. Med.* **121**, 173–177
- Cuatrecasas, P. (1971) *J. Biol. Chem.* **246**, 6532–6542
- Cuatrecasas, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 318–322
- Cuatrecasas, P. (1974) *Annu. Rev. Biochem.* **43**, 169–214
- Cuatrecasas, P. & Hollenberg, M. D. (1976) *Adv. Protein Chem.* **30**, 252–450
- Dang Vu, T. K. & Bénard, B. (1978) *Ann. Endocrinol.* **39**, 451–462
- De Kretser, D. M., Catt, K. J., Burger, H. G. & Smith, G. C. (1969) *J. Endocrinol.* **43**, 105–111
- Donatsch, P. & Richardson, B. (1975) *J. Endocrinol.* **66**, 101–106
- Du Caju, M., Postel-Vinay, M. C. & Desbuquois, B. (1978) *Arch. Fr. Pédiatr.* **35** Suppl., 102–114
- Evan, A. P., Palmer, G. C., Lucci, M. S. & Solomon, S. (1977) *Nephron* **18**, 266–276
- Frantz, W. L., MacIndoe, J. H. & Turkington, R. W. (1974) *J. Endocrinol.* **60**, 485–497
- Herington, A. C., Veith, N. M. & Burger, H. G. (1976) *Biochem. J.* **158**, 61–69
- Horrobin, D. F., Lloyd, I. J., Lipton, A., Burstyn, P. G., Durkin, N. & Muiruri, K. L. (1971) *Lancet* **ii**, 352–354
- Horrobin, D. F., Manku, M. S. & Burstyn, P. G. (1973) *J. Endocrinol.* **56**, 343–344
- Hughes, J. P. (1979) *Endocrinology* **105**, 414–420
- Johnson, V. & Maack, T. (1977) *Am. J. Physiol.* **233**, F185–F196
- Kahn, C. R. (1976) *J. Cell. Biol.* **70**, 261–286
- Kelly, P. A., Posner, B. I., Tsushima, T. & Friesen, H. G. (1974) *Endocrinology* **95**, 532–539
- Kleinberg, D. L. & Frantz, A. G. (1971) *J. Clin. Invest.* **50**, 1557–1568
- Lees, P., Lockett, M. F. & Roberts, C. N. (1964) *J. Physiol. (London)* **171**, 397–402
- Li, C. H. & Papkoff, H. (1956) *Science* **124**, 1293–1294
- Lockett, M. F. (1965) *J. Physiol. (London)* **181**, 192–199
- Lockett, M. F. & Nail, B. (1965) *J. Physiol. (London)* **180**, 147–156
- Lockett, M. F. & Roberts, C. N. (1963) *J. Physiol. (London)* **169**, 879–888
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Manni, A., Chambers, M. J. & Pearson, O. H. (1978) *Endocrinology* **103**, 2168–2171
- Marshall, S., Gelato, M. & Meites, J. (1975) *Proc. Soc. Exp. Biol. Med.* **149**, 185–188
- Marshall, S., Kledzik, G. S., Gelato, M., Campbell, G. A. & Meites, J. (1976) *Steroids* **27**, 187–195
- Marshall, S., Huang, H. H., Kledzik, G. S., Campbell, G. A. & Meites, J. (1978) *Endocrinology* **102**, 869–875
- Marshall, S., Bruni, J. F. & Meites, J. (1979) *Endocrinology* **104**, 390–395
- Mess, B. & Hámori, J. (1965) *Acta Histochem.* **20**, 143–148
- Mountjoy, K., Cowden, E. A., Dobbie, J. W. & Ratcliffe, J. G. (1980) *J. Endocrinol.* **87**, 47–54
- Posner, B. I. (1975) *Can. J. Physiol. Pharmacol.* **53**, 689–703
- Posner, B. I. (1976) *Endocrinology* **98**, 645–654

- Posner, B. I., Kelly, P. A., Shiu, R. P. C. & Friesen, H. G. (1974) *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
- Rajaniemi, H., Oksanen, A. & Vanha-Perttula, T. (1974) *Horm. Res.* **5**, 6–20
- 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. D., Dellacha, J. M. & Paladini, A. C. (1977) *Mol. Cell. Biochem.* **16**, 87–96
- Sakai, S., Kohnoto, K. & Johke, T. (1975) *Endocrinol. Jpn.* **22**, 379–387
- Salmon, S., Utiger, R., Parker, M. & Reichlin, S. (1962) *Endocrinology* **70**, 459–464
- Sánchez, S. H., Vidal, J. C. & Paladini, A. C. (1979) *Arch. Biol. Med. Exp.* **12**, 284
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Shiu, R. P. C. & Friesen, H. G. (1974) *J. Biol. Chem.* **249**, 7902–7911
- Silverstein, A. M. & Richards, J. F. (1979) *Biochem. J.* **178**, 743–751
- Sonenberg, M., Money, W. L., Dorans, J. F., Lucas, V. & Bourque, L. (1954) *Endocrinology* **55**, 709–720
- Stacy, B. D., Wallace, A. L. C., Gemmell, R. T. & Wilson, B. W. (1976) *J. Endocrinol.* **68**, 21–30
- Tsushima, T. & Friesen, H. G. (1973) *J. Clin. Endocrinol. Metab.* **37**, 334–337
- Turyn, D., Santomé, J. A. & Dellacha, J. M. (1976) *Acta Physiol. Lat. Am.* **26**, 395–402
- Wallace, A. L. C., Stacy, B. D. & Thorburn, G. D. (1972) *Pflügers Arch.* **331**, 25–37
- Wallin, J. D. & Lee, P. A. (1976) *Am. J. Physiol.* **230**, 1524–1530
- Wallis, M. & Cadman, H. F. (1981) *Biochem. Soc. Trans.* **9**, 33–35
- Waters, M. J. & Friesen, H. G. (1979) *J. Biol. Chem.* **254**, 6815–6825