The specificity of binding of growth hormone and prolactin to purified plasma membranes from pregnant-rabbit liver

Cathryn F. WEBB, Hilary F. CADMAN* and Michael WALLIS

Biochemistry Laboratory, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, Sussex, U.K.

The binding of ^{125}I -labelled human growth hormone (hGH) to a purified plasma membrane preparation from the liver of pregnant rabbit, and to receptors solubilized from this fraction with Triton X-100, was dependent on time, temperature, the cations used and the receptor concentration. Solubilization did not affect the binding properties of the receptors at low concentrations of Triton X-100. Some somatogenic hormones, such as bovine GH, and some lactogenic hormones, such as ovine prolactin, displaced ^{125}I -labelled hGH from purified plasma membranes and solubilized receptor preparations, but GHs and prolactins from various other species were rather ineffective. The results indicate that although there are binding sites for hGH in these pregnant rabbit liver membranes, few of these are specifically somatogenic or lactogenic. The binding properties of the purified plasma membranes are similar to those of a microsomal preparation studied previously, suggesting that the complex nature of the binding of hGH is not due to the heterogeneity of cellular membranes used to study binding, but is a property of the receptors associated with plasma membranes.

INTRODUCTION

Specific binding sites for lactogenic and somatogenic hormones are found in various vertebrate tissues, but the relationship between these and the biological actions of prolactin, growth hormone (GH; somatotropin) and related hormones remains unclear in many cases. The most important target for lactogenic hormones in mammals is the mammary gland, but the prime target tissue for somatogenic hormones is less well defined. It is clear, however, that many of the anabolic effects of GH are mediated by somatomedins, a major production site of which is the liver (McConaghey & Sledge, 1970) and this, and other direct actions that have been described, suggest that the liver represents a major target organ of GH action. Interpretation of the data on somatogenic and lactogenic binding sites in liver has been complicated by the fact that human GH, which has been the most widely used labelled ligand, has lactogenic as well as somatogenic activity and therefore binds to both lactogenic and somatogenic receptors.

A high level of binding sites for hGH has been detected in the liver of the pregnant rabbit (Tsushima & Friesen, 1973), but there has been some disagreement about the nature of these sites. Tsushima & Friesen (1973) considered them primarily somatogenic, but later workers provided evidence for lactogenic receptors in rabbit liver also (Posner *et al.*, 1974; Parke & Forsyth, 1975; Waters & Friesen, 1979; Fix *et al.*, 1981; Haeuptle *et al.*, 1983). Our previous work (Cadman & Wallis, 1980, 1981; Wallis *et al.*, 1984) and that of Hughes (1979) and Fix *et al.* (1981) suggests that the situation is complex and cannot be explained entirely in terms of a mixture of distinct somatogenic and lactogenic receptors. Thus, most ^{125}I -labelled hGH can be displaced by both lactogenic and somatotropic hormones, indicating the

presence of a 'predominant receptor' with a specificity that does not correlate well with biological activity. These studies suggest that the liver membranes may contain at least three types of receptor: a specific somatogenic receptor, with high affinity for rabbit GH, a specific lactogenic receptor, and a third rather non-specific receptor which binds various GHs and prolactins, but has a low affinity for the rabbit hormones.

Much of the previous work carried out on rabbit liver receptors has employed microsomal membrane preparations; these include plasma membrane together with various other types of cellular membrane. The complex nature of the results obtained with this system could be partly accounted for by the observation that many GH and prolactin receptors are located intracellularly (Bergeron *et al.*, 1978), since intracellular and plasma-membrane bound receptors might be different in kind. It seemed worthwhile, therefore, to reinvestigate the properties of the receptors with the use of purified plasma membranes, in order to determine whether the resulting increase in membrane homogeneity resulted in a corresponding decrease in apparent receptor heterogeneity. The results of this investigation are presented here.

MATERIALS AND METHODS

Materials

hGH was a gift from the National Institute of Biological Standards and Control, Hampstead, London, U.K. Bovine GH (NIH-GH-B16), ovine GH (NIH-GH-S9), rat GH (GH-B6/AFP-1765-B), rabbit GH (AFP-684-C), canine GH (AFP-1983-B), mouse GH (AFP-689-B), human prolactin (NIH-hPRL-VLS-4), bovine prolactin (NIH-P-B3), ovine prolactin (NIAMDD-

Abbreviations used: GH, growth hormone (somatotropin); hGH, human growth hormone; BSA, bovine serum albumin.

* Present address: Scottish Antibody Production Unit, Glasgow and West Scotland Blood Transfusion Service, Law Hospital, Carluke, Lanarkshire ML8 4BR, U.K.

oPRL-14), rat prolactin (PRL-B-2; AFP-2860-D), rabbit prolactin (AFP-1974-C), canine prolactin (AFP-2451-B) and mouse prolactin (AFP-1411-E) were gifts from Dr. A. F. Parlow, Dr. A. E. Wilhelmi and The National Institute of Arthritis, Diabetes and Digestive and Kidney Disorders, NIH, Bethesda, MD, U.S.A. Human placental lactogen was a gift from Dr. K. Humphreys of Sigma (London). Porcine somatotropin (28B-2350), insulin (66C-6395), bovine serum albumin (BSA) (Cohn fraction V) and merthiolate (thimerosal) were purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K. Sephadex G-50 (fine grade) and Sephadex G-100 (fine grade) were obtained from Pharmacia, Hounslow, Middlesex, U.K. Na¹²⁵I (carrier-free; IMS 30) was from Amersham International, Amersham, Bucks., U.K. Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril) was from Pierce & Warriner (U.K.), Chester, Cheshire, U.K. Poly(ethylene glycol) 6000 and Triton X-100 were obtained from BDH Chemicals, Poole, Dorset, U.K. Pregnant New Zealand White rabbits were from Buxted Rabbits, Buxted, Sussex, U.K.

Iodination of hormones

¹²⁵I-labelled hGH was prepared by the Iodogen method of Fraker & Speck (1978), as used by Cadman & Wallis (1981). The labelled hormone was purified by gel filtration on columns of Sephadex G-50, and then G-100, as described previously (Cadman & Wallis, 1981); after purification the labelled hGH was in 0.025 M-Tris/HCl buffer, pH 7.6, containing 0.1% BSA and 0.6 mM-merthiolate unless otherwise stated. The specific activity of the ¹²⁵I-labelled hormone was 50–90 μ Ci/ μ g of hormone.

Preparation of purified plasma membranes

Plasma membranes from the liver of pregnant rabbits were isolated by the method of Neville (1968) as far as step II of the procedure, with scaling up as described by Goldstein & Blecher (1976) in order to increase the yield. The liver (approx. 100 g) of a 26–29 day pregnant rabbit was divided into 10 g portions and chopped finely in a beaker; 35 ml of medium (1 mM-NaHCO₃) was added to each portion and the suspension was homogenized with eight strokes of a loose-fitting Dounce homogenizer. Two portions were combined, made up to 500 ml with medium, stirred for 5 min, and then filtered through two

layers of cheesecloth. The filtrate was centrifuged (1500 g, 10 min, 4 °C) and the supernatant was discarded. The homogenization/centrifugation step was repeated for all portions of the liver, and the pellets were pooled and rehomogenized with three strokes of a Dounce homogenizer.

The sucrose concentration of the suspension was adjusted to $44.0 \pm 0.1\%$ (w/w) with a 70% sucrose solution, and 24 ml of tissue suspension (about 25% of the total) was placed in each of four or five 35 ml centrifuge tubes and overlaid with 11 ml of $42.3 \pm 0.1\%$ (w/w) sucrose. The tubes were centrifuged (95000 g, 2 h, 4 °C) and the 'floats' containing the plasma membranes were removed, made up to 35 ml with ice-cold medium, mixed well and centrifuged (40000 g, 30 min, 4 °C). The supernatant was discarded and the pellet (plasma membrane preparation) was resuspended in an equal volume of medium. Samples were taken at various stages through the procedure, for binding studies and enzyme marker assays.

Microsomal membranes were prepared as described by Cadman & Wallis (1981).

Solubilization of receptors

Purified plasma membrane preparations were solubilized by the method of Shiu & Friesen (1974) and Herington & Veith (1977) as modified by Cadman & Wallis (1981). Purified plasma membranes were diluted with 'assay buffer' (0.025 M-Tris/HCl, pH 7.6, containing 0.1% BSA, 0.6 mM-merthiolate and 10 mM-CaCl₂) to a protein concentration of 10 mg/ml, and Triton X-100 was added to a final concentration of 0.5% (v/v). The suspension was then stirred for 30 min at 20–25 °C and centrifuged at 200000 g for 2 h at 4 °C. The supernatant ('solubilized receptors') was stored at –70 °C.

Binding studies

The incubation procedure was identical for purified plasma membranes and solubilized plasma-membrane receptors. Except where indicated, all dilutions of receptor preparation, standards, and labelled hormone were carried out in 'assay buffer' (see above). Normally each assay tube contained 200 μ l of assay buffer, 100 μ l of plasma membranes (50 μ g of protein) or solubilized receptors (10 μ g of protein), 100 μ l of unlabelled hormone ranging from 0.32 to 1000 ng/tube, and 100 μ l

Table 1. Distribution of enzyme activity in subcellular fractions from the plasma membrane and microsomal membrane preparations from rabbit liver

Cell fraction	Enzyme activity (μ g of phosphate liberated/mg of protein)	
	(Na ⁺ + K ⁺)-ATPase*	Glucose-6-phosphatase†
Plasma membrane preparation		
Filtered homogenate	–	90 $\pm$ 2
First pellet	26 (20–33)	259 $\pm$ 3
Final pellet (plasma membrane)	255 (180–330)	23 $\pm$ 0.4
Microsomal membrane preparation		
Filtered homogenate	54 (30–75)	85 $\pm$ 3
Final homogenate (microsomes)	21 (0–58)	141 $\pm$ 5

* Mean, with maximum range in parentheses.

† Mean $\pm$ S.E.M.

of ^{125}I -labelled hGH (approx. 30 000 c.p.m./tube). Tubes were mixed and incubated for 20–24 h at 4 °C. The assay using plasma membrane receptors was terminated by the addition of 2 ml of ice-cold 0.25 M-sodium acetate/acetic acid buffer, pH 5.4, containing 0.1% BSA and 0.6 mM-merthiolate. The assay using solubilized receptors was terminated by adding 0.5 ml of ice-cold sodium phosphate buffer (0.1 M- NaH_2PO_4 , adjusted to pH 7.5 with NaOH), followed by 1 ml of ice-cold 25% poly(ethylene glycol) 6000. The tubes were mixed thoroughly and allowed to stand at 4 °C for 30 min before centrifugation (1000 g, 25 min, 4 °C). Supernatants were removed by aspiration and precipitates subjected to γ -radiation counting using a Nuclear Chicago model 1195 counter; counting efficiency for ^{125}I was 50–55%. Binding of labelled hGH that occurred in the presence of excess (1 μg) unlabelled hormone was considered to be non-specific and was subsequently subtracted from the total binding to give the specific binding.

Enzyme marker assays

Marker enzyme assays were used to characterize the plasma membrane preparations. The method of Wheeler *et al.* (1975) was used to assay ($\text{Na}^+ + \text{K}^+$)-ATPase and the method of Hübscher & West (1965) was used to assay glucose-6-phosphatase. The amount of inorganic phosphate liberated in these assays was determined by the method of LeBel *et al.* (1978).

RESULTS

Comparison of enzyme activities of subcellular fractions from a plasma membrane preparation and a microsomal membrane preparation

Assays for the enzymes ($\text{Na}^+ + \text{K}^+$)-ATPase (marker for plasma membranes) and glucose-6-phosphatase (marker for endoplasmic reticulum) were carried out on various subcellular fractions from the plasma membrane preparation and a microsomal membrane preparation (Tsushima & Friesen, 1973; Cadman & Wallis, 1981) from pregnant rabbit liver. The results from one experiment are shown in Table 1. These and results from similar experiments confirm that the 'plasma membrane preparation' was indeed enriched with regard to plasma membranes, with an 11-fold increase in the concentrations of ($\text{Na}^+ + \text{K}^+$)-ATPase and an 11-fold decrease in the concentration of glucose-6-phosphatase, compared with the first pellet. On the other hand, the microsomal preparation showed a 2-fold decrease in the activity of ($\text{Na}^+ + \text{K}^+$)-ATPase and a 1.6-fold increase of activity of glucose-6-phosphatase, compared with the filtered homogenate.

The results indicate that there may be a small amount of contamination of the plasma membrane preparation by endoplasmic reticulum. However, it is clear that the enrichment in plasma membranes in this preparation is very substantial, compared with the microsomal preparation that we have used previously.

Effects of time and temperature on hormone binding

Specific binding of ^{125}I -labelled hGH to purified plasma membranes was time- and temperature-dependent (Fig. 1). Non-specific binding was constant after 8 h at 4 °C and 25 °C. Binding to the purified receptors was more rapid at 25 °C than at 4 °C. Equilibrium was

Fig. 1. Effect of time and temperature on the binding of ^{125}I -labelled hGH to purified plasma membranes

^{125}I -labelled hGH (approx. 34 nCi/tube, equivalent to 30 000 c.p.m.) was incubated with receptor preparation (20 μg of protein/tube) at 4 °C (●, total binding; ○, non-specific binding) or at 25 °C (■, total binding; □, non-specific binding). Each value represents the mean of triplicate determinations.

approached by 12 h at 25 °C but required at least 26 h at 4 °C. The mean specific binding achieved at 26 h at 4 °C was $23.0 \pm 0.7\%$ of total radioactivity added (results are mean $\pm$ S.E.M. of triplicate determinations) and at 25 °C it was $35.2 \pm 0.7\%$. Non-specific binding was slightly lower at 4 °C ($8.61 \pm 0.04\%$) than at 25 °C ($9.45 \pm 0.17\%$).

Effect of receptor concentration on binding of human growth hormone

Fig. 2 shows the effect of increasing concentration of plasma membrane receptors and solubilized plasma membrane receptors on the specific binding of ^{125}I -labelled hGH. Specific binding increased up to a maximum with 60 μg of protein/tube for the purified plasma membranes (Fig. 2a) and up to a maximum with 20 μg of protein/tube for solubilized plasma membranes (Fig. 2b). Maximum specific binding was 40% of the total radioactivity added for plasma membrane receptors and 36% for solubilized receptors. Maximum specific binding varied from 30 to 50% in different experiments, due to variations in the receptor preparations and labelled hormone.

Effect of cations on hormone binding

Fig. 3 illustrates the effect of receptor concentration on the binding of ^{125}I -labelled hGH in conjunction with different cations. The divalent cations (Ca^{2+} , Mg^{2+}) stimulate hGH binding, whereas, in the presence of the monovalent cation, Na^+ , binding was not significantly greater than in the absence of metallic cations. Ca^{2+} increased binding most, giving a maximum specific binding of 31.2% with 100 μg of protein/tube. At 100 μg of protein/tube binding was 22% with MgCl_2 , 15% with

Fig. 2. Effect of receptor concentration on the binding of ^{125}I -labelled hGH to (a) purified plasma membranes, and (b) solubilized plasma membranes

^{125}I -labelled hGH (approx. 34 nCi/tube) was incubated for 22 h at 4 °C with increasing concentrations of receptor preparation (●, total binding; ○, non-specific binding). Each value represents the mean of triplicate determinations.

NaCl and 13% with no metallic cations present (non-specific binding was 9–10% of the total radioactivity added). Thus, CaCl_2 gave a greater increase in binding than did MgCl_2 , and this was due mainly to increased specific binding.

Specificity of the receptors

Figs. 4 and 5 show the competition between ^{125}I -labelled hGH and various unlabelled hormones (displacement curves) for binding to plasma membrane-associated receptors. The displacement curves for solubilized plasma

Fig. 3. Effects of cation addition on the binding of ^{125}I -labelled hGH; effect of increasing receptor concentration

^{125}I -labelled hGH (approx. 33 nCi/tube) was incubated for 22 h at 4 °C with increasing concentrations of receptor preparation in the presence of 10 mM- CaCl_2 (●), 10 mM- MgCl_2 (○), 10 mM- NaCl (▼) or no added metallic cation (▽). Specific binding (% of total radioactivity added) was calculated.

Fig. 4. Specificity of displacement of ^{125}I -labelled hGH from purified plasma membranes by various GHs

^{125}I -labelled hGH (34 nCi/tube) was incubated with receptor preparation (50–60 µg of protein/tube) in the presence of increasing concentrations of unlabelled hGH (○), ovine GH (●), bovine GH (■), rat GH (□), pig GH (▼), dog GH (▲), mouse GH (△) and insulin (▽). X, no additions. Each value represents the mean of triplicate determinations.

Fig. 5. Specificity of displacement of ^{125}I -labelled hGH from purified plasma membranes by various prolactins

^{125}I -labelled hGH (34 nCi/tube) was incubated with receptor preparation (50–60 μg of protein/tube) in the presence of increasing concentrations of unlabelled human GH (●), bovine prolactin (▽), ovine prolactin (■), human prolactin (△), mouse prolactin (▼), rabbit prolactin (□), dog prolactin (○) and rat prolactin (▲). X, no additions. Each value represents the mean of triplicate determinations.

membranes (not shown) were very similar, suggesting that solubilization does not significantly affect the binding properties of the receptors (provided the final concentration of Triton X-100 in the assay tubes is below 0.05%). Bovine and ovine GH displaced ^{125}I -labelled hGH with potency similar to that of unlabelled hGH (Fig. 4). Pig, rat, rabbit, dog and mouse GHs also competed for receptors but with a lower potency than hGH. Insulin did not compete with ^{125}I -labelled hGH for binding sites. Bovine and ovine prolactin competed for binding sites for ^{125}I -labelled hGH (Fig. 5), the bovine hormone being more potent than ovine prolactin, which is surprising since bovine and ovine prolactin have very similar sequences (Wallis, 1978). Human prolactin displaced ^{125}I -labelled hGH to some extent, while mouse, rabbit, canine and rat prolactin and human placental lactogen (results not shown) competed poorly with ^{125}I -labelled hGH.

DISCUSSION

Previous work using membrane-bound 'receptors' for hGH indicated that the liver of the pregnant rabbit is a particularly rich source of such material (Tsushima & Friesen, 1973). Subsequently, detailed studies showed that specificity for the binding of GHs and prolactins from various species to such receptors does not accord closely with either the growth-promoting or lactogenic properties of the hormones (Hughes, 1979; Cadman & Wallis, 1981; Fix *et al.*, 1981; Wallis & Cadman, 1981; Haeuptle *et al.*, 1983). Such studies suggest that the liver contains several different receptors for hormones of the GH/prolactin family, only some of which mediate growth-promoting or lactogenic actions. However, much of the work carried out with receptors of this kind has used microsomal membrane preparations which include several different types of cell membrane in addition to plasma membranes. Many of the receptors for GH and prolactin in liver appear to be located intracellularly (Bergeron *et al.*, 1978) and it is therefore likely that microsomal membranes will include both plasma membrane-bound receptors and receptors associated with

Table 2. Summary of the binding potencies of somatogenic hormones determined by radioreceptor assay and bioassay

Hormone	Radioreceptor assay*		Bioassay† potency (i.u./mg)
	Amount of hormone needed for 50% maximal displacement (ng/tube)	Relative potency (compared with hGH)	
Human GH	5.62	1	Approx. 2.5
Ovine GH (NIH GH-S9)	9.44	0.59	1.7
Bovine GH (NIH GH-B16)	10	0.56	0.81
Canine GH (NIH AFP-1983-B)	53	0.11	Unspecified
Rat GH (NIH GH-B6/AFP-1765-B)	63	0.09	1.7
Mouse GH (NIH AFP-689-B)	66.80	0.08	Unspecified
Pig GH (Sigma 28B-2350)	177.80	0.03	0.5

* Based on data of Fig. 4.

† Based on information provided by suppliers; determined using promotion of weight gain in the hypophysectomized rat.

various intracellular organelles. This potential heterogeneity could contribute to the complex nature and binding properties of the microsomal receptors, and it therefore seemed worthwhile to investigate the properties of the receptors in plasma membranes purified from the liver of pregnant rabbits, with particular emphasis on the ability of GHs and prolactins from a wide range of species to displace ^{125}I -labelled hGH. Some previous work with purified plasma membranes has indicated that these have similar binding properties to the microsomal membranes (Fix *et al.*, 1981), but detailed specificity studies were not carried out.

Binding of ^{125}I -labelled hGH to the liver plasma membrane preparation was dependent on time and temperature and on the concentration of the receptor preparation, as was found using microsomal membranes (Cadman & Wallis, 1981). Binding of ^{125}I -labelled hGH was divalent cation dependent; Ca^{2+} was somewhat more effective than Mg^{2+} and was therefore used in the assay buffer in all the other experiments described in this paper. Fix *et al.* (1981) obtained similar results and also suggested that hGH and ovine prolactin are dependent on divalent cations for binding, but that bovine and rat GH are not, an observation which accords with the possibility that hGH and bovine or rat GHs bind to different sites. Solubilization of the purified plasma membranes using the detergent Triton X-100 did not alter the binding properties of the receptors, as long as the final concentration of the detergent was less than 0.05%; higher concentrations of Triton X-100 affect the stability of ovine prolactin.

Comparison of the potencies of GHs from various species with regard to ability to displace labelled hGH from receptors suggests that there are two distinct groups. The ruminant GHs (bovine and ovine) are almost as effective as the human hormone in this respect, but GHs from most other non-primate mammals are much less effective (Table 2). To some extent this reflects structural relationships: thus ovine and bovine GH are very similar, and rather different in their primary structure from GHs of pig and rat, and probably other non-primate GHs (Wallis, 1981). Nevertheless, hGH is very different from the bovine hormone, despite the similarities in receptor-binding potency. The relative receptor-binding potencies differ markedly from the reported growth-promoting activity of the hormone preparations used (Table 2). The potencies of prolactins obtained from conventional bioassays also do not correspond well with the radioreceptor assay potencies. It should be noted, however, that biological activity *in vivo* may not reflect solely the potency of the hormone at its receptor. Other considerations, including the half-life of the hormone in the circulation, may affect the activity, and in the absence of information about clearance rates of the different hormones studied, conclusions about the discrepancies between growth-promoting activity and receptor-binding activity must be tentative.

The results obtained here with receptors associated with plasma membranes are very similar to those obtained previously in our laboratory using a crude microsomal membrane preparation (Cadman & Wallis, 1981). Thus, the complexity of the microsomal system does not reflect the heterogeneity of the membrane preparation used, but reflects the nature of the plasma membrane-associated receptors themselves (though the plasma membrane preparation may, of course, contain

some contamination with other types of membrane as indicated by the results from the enzyme marker assay).

Overall, the results presented here indicate that, as was concluded for the microsomal membrane preparation, the bulk of the binding sites for hGH in the pregnant rabbit liver do not mediate the growth-promoting actions of GH. Such a conclusion accords well with the work of Hughes (1979), who suggested the presence of at least two types of somatogenic binding site in rabbit liver, and of Fix *et al.* (1981) who found binding of hGH in plasma membranes from rabbit liver that could not be explained by the lactogenic or somatogenic potential of the hormone. Heterogeneity of the GH receptors in rabbit liver has also been suggested by Simpson *et al.* (1983) and Barnard *et al.* (1985), using monoclonal antibodies to the receptors, and Hughes *et al.* (1983), on the basis of cross-linking studies. Haeuptle *et al.* (1983) found that photoaffinity labelling could detect one major and several minor components corresponding to a GH receptor.

Several authors have advocated recently the importance of using homologous systems to investigate receptor binding by members of the GH/prolactin family (Haro *et al.*, 1984; Hung *et al.*, 1985). There is no doubt that use of the heterologous system described here (human GH and rabbit membranes) leads to complexities that may be partly avoided by concentration on a homologous system. Nevertheless, use of ^{125}I -labelled hGH has led to the identification of binding sites in the rabbit liver that would probably not have been detected if labelled rabbit GH had been used. The biological role of these binding sites remains to be elucidated; possibly they mediate the actions of another member of the growth hormone-prolactin family (such as placental lactogen), or are involved in mediating degradation and removal of the hormone from the circulation.

We thank the MRC and SERC for research support, Dr. A. Parlow, Dr. A. E. Wilhelmi and the National Pituitary Agency for gifts of hormones, and Mrs. Eileen Willis for skilled preparation of the manuscript.

REFERENCES

- Barnard, R., Bundesen, P. G., Rylatt, D. B. & Waters, M. J. (1985) *Biochem. J.* **231**, 459–468
- Bergeron, J. J. M., Posner, B. I., Josefsberg, Z. & Sikstrom, R. (1978) *J. Biol. Chem.* **253**, 4058–4066
- Cadman, H. F. & Wallis, M. (1980) *J. Endocrinol.* **87**, 53P–54P
- Cadman, H. F. & Wallis, M. (1981) *Biochem. J.* **198**, 605–614
- Fix, J. A., Leppert, P. & Moore, W. V. (1981) *Horm. Metab. Res.* **13**, 510–515
- Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–857
- Goldstein, S. & Blecher, M. (1976) in *Methods in Receptor Research*, part I, (Blecher, M., ed.), p. 119–142, Dekker, New York
- Haeuptle, M.-T., Aubert, M. L., Djiane, J. & Kraehenbuhl, J.-P. (1983) *J. Biol. Chem.* **258**, 305–314
- Haro, L. S., Collier, R. J. & Talamantes, F. J. (1984) *Mol. Cell Endocrinol.* **38**, 109–116
- Herington, A. C. & Veith, N. M. (1977) *Endocrinology* (Baltimore) **101**, 984–987
- Herington, A. C., Veith, N. & Burger, H. G. (1976) *Biochem. J.* **158**, 61–69
- Hübscher, G. & West, G. R. (1965) *Nature (London)* **205**, 799–800
- Hughes, J. P. (1979) *Endocrinology* (Baltimore) **105**, 414–420

- Hughes, J. P., Simpson, J. S. A. & Friesen, H. G. (1983) *Endocrinology* (Baltimore) **112**, 1980–1985
- Hung, C. H., Kover, K. & Moore, W. V. (1985) *Mol. Cell. Endocrinol.* **39**, 189–196
- LeBel, D., Poirier, G. G. & Beaudoin, A. R. (1978) *Anal. Biochem.* **85**, 86–89
- McConaghey, P. & Sledge, C. B. (1970) *Nature* (London) **225**, 1249–1250
- Neville, D. M. (1968) *Biochim. Biophys. Acta* **154**, 540–552
- Parke, L. A. & Forsyth, I. A. (1975) *Endocr. Res. Commun.* **2**, 137–149
- Posner, B. I., Kelly, P. A., Shiu, R. P. C. & Friesen, H. G. (1974) *Endocrinology* (Baltimore) **95**, 521–531
- Shiu, R. P. C. & Friesen, H. G. (1974) *J. Biol. Chem.* **249**, 7902–7911
- Simpson, J. S. A., Hughes, J. P. & Friesen, H. G. (1983) *Endocrinology* (Baltimore) **112**, 2137–2141
- Tsushima, T. & Friesen, H. G. (1973) *J. Clin. Endocrinol. Metab.* **37**, 334–337
- Wallis, M. (1978) in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins* (Weinstein, B., ed.), vol. 5, pp. 213–320, Dekker, New York
- Wallis, M. (1981) *J. Mol. Evol.* **17**, 10–18
- Wallis, M. & Cadman, H. F. (1981) *Biochem. Soc. Trans.* **9**, 33–35
- Wallis, M., Cadman, H. F. & Learoyd, C. F. (1984) in *Methodological Surveys in Biochemistry and Analysis*, Vol. 13: *Investigation of Membrane-Located Receptors* (Reid, E., Cook, G. M. W. & Morré, D. J., eds.), pp. 263–275, Plenum Press, New York
- Waters, M. J. & Friesen, H. G. (1979) *J. Biol. Chem.* **254**, 6815–6825
- Wheeler, K. P., Walker, J. A. & Barker, D. M. (1975) *Biochem. J.* **146**, 713–722

Received 5 December 1985/24 January 1986; accepted 10 February 1986