Identification of a protein from rat liver cytosol that enhances activation of the glucocorticoid receptor

John L. TYMOCZKO*, Shawn AHERN, Alison L. UNGER, Jennifer L. COLBY and Georgia EDE
Department of Biology, Carleton College, Northfield, MN 55057-4025, U.S.A.

We have identified a factor from rat liver cytosol that enhances the DNA–cellulose-binding ability of the glucocorticoid receptor and lowers the sedimentation value from 9–10 S to 4–5 S. Cytosol is prepared in the presence of molybdate, and unactivated receptor is isolated by chromatography on DEAE–cellulose in the presence of molybdate. This receptor sediments at 9–10 S and has little affinity for DNA. If the molybdate is removed and the receptor is incubated at 25 °C with the low-salt wash of the DEAE–cellulose column, DNA binding is enhanced by 50–600 % relative to controls incubated with buffer only. In addition, the factor present in the low-salt wash converts the 9–10 S receptor into a mixture of 5 S and 4 S forms. The factor must be present during the incubation in order to exert its maximal effect. Factor added after the incubation has only marginal effects on the DNA-binding ability of the receptor, indicating that the factor does not increase the DNA-binding ability of activated receptor. Moreover, the factor is significantly less effective on receptor that has been activated before incubation with the factor. These results suggest that the factor acts as an activation enhancer. Preliminary characterization indicates that the activation enhancer is a trypsin-sensitive protein of approx. 70000 Da, whose activation-enhancing properties are inhibited by ATP. RNAase A, which has effects similar to those described above on the 7–8 S receptor, does not mimic the effects of the activation enhancer on the 9–10 S receptor.

INTRODUCTION

The mechanism by which the glucocorticoid–receptor complex is converted from the unactivated 9–10 S form into the activated 4–5 S form remains a mystery, despite substantial efforts to understand this process. We have previously presented evidence that the 9–10 S form of the receptor can be converted into a low-salt 7–8 S form which is associated with RNA (Tymoczko & Phillips, 1983; Tymoczko *et al.*, 1984). Although the 7–8 S RNA-associated receptor can bind to DNA several times more efficiently than the unactivated glucocorticoid–receptor complex, the removal of the RNA by RNAase leads to further enhancement of DNA binding as well as conversion into a 5 S form (Tymoczko *et al.*, 1984, 1986; Rossini, 1985). Whether RNA is associated with the glucocorticoid–receptor complex while it is in the RNAase-resistant 9–10 S form is not clear. There is a report that the 9–10 S receptor from AtT-20 cells is free of RNA and becomes associated with RNA on conversion into the 6 S form (Kovacic-Milivojevic & Vedeckis, 1986). However, there are also reports that a highly purified 9–10 S receptor preparation from rat liver contains RNA (Webb *et al.*, 1986). Furthermore, we have shown that, when 9–10 S receptor is isolated with monoclonal antibody to the receptor, distinct species of RNA are found in the receptor preparation (Unger *et al.*, 1987).

Our previous work demonstrated that, to convert 9–10 S receptor into a DNA-binding form, a low-molecular-mass activation inhibitor must be removed from the receptor (Anderson and Tymoczko, 1985). Upon removal of this factor, the 7–8 S form of the receptor is seen, which can be digested with RNAase A

or RNAase T₁ (Tymoczko & Phillips, 1983). Work by Litwack and collaborators has identified a protein factor that can stimulate activation of purified 9–10 S receptor (Schmidt *et al.*, 1985) and suggested that this protein may be an RNAase (Webb & Litwack, 1986).

Here we describe a protein factor from rat liver cytosol that is able to convert the 9–10 S receptor directly into a mixture of 5 S and 4 S forms without formation of the 7–8 S form of the receptor. The factor also enhances the ability of the glucocorticoid–receptor complex to interact with DNA–cellulose. This factor is apparently not an RNAase, as RNAase A is not able to mimic its effects. This factor behaves as an activation enhancer in that its ability to stimulate DNA binding is most profound with unactivated glucocorticoid–receptor complex.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade. [6,7(n)-³H]-Dexamethasone (49 Ci/mmol), [methyl-¹⁴C]ovalbumin (11.2 Ci/mg) and [¹⁴C]formaldehyde (10 mCi/mmol) were purchased from Dupont–New England Nuclear. Chymotrypsin, trypsin, aldolase and lima-bean trypsin inhibitor were purchased from Sigma. RNAase A was obtained from Worthington.

Preparation of receptor

Adrenalectomized rats of the Sprague–Dawley strain were killed by cervical dislocation 4–21 days after surgery. All procedures were performed at 0–4 °C unless otherwise specified. The livers were perfused *in situ* with 0.145 M-NaCl (0–4 °C) and homogenized in 1 vol. of 20 mM-Tris/

* To whom all correspondence should be addressed.

HCl (pH 7.5)/1.5 mM-EDTA/1 mM-dithiothreitol/10 % (v/v) glycerol / 10 mM-sodium molybdate (TEDGM). The homogenate was centrifuged at 600 *g* for 10 min, and the supernatant was made to 28 nM with [6,7(n)-³H]dexamethasone and incubated on ice for 1 h. The sample was then centrifuged at 105 000 *g* for 1 h, and the supernatant was used as the source of receptor. Specific macromolecular-bound steroid was at least 90 % of the total dexamethasone radioactivity as determined by the dextran-charcoal technique (Beato & Feigelson, 1972).

To prepare 9–10 S receptor and the activation factor, a 2 ml portion of cytosol was layered on an 8 ml DEAE-cellulose (Whatman) column. The column was first washed with 20 ml of 0.05 M-KCl/TEDGM, followed by 20 ml of 0.2 M-KCl/TEDGM. The activation enhancer was eluted in the low-salt wash, whereas the unactivated receptor was eluted with the high-salt wash. Both samples were dialysed for 1 h against TEDG (TEDGM minus the molybdate) to remove the KCl and molybdate. The DEAE-cellulose procedure resulted in a 7-fold purification of the receptor, with a 50 % yield. In some instances, as described in the text and Figure legends, a 10 mM-MgCl₂/TEDGM wash was included after the 0.05 M-KCl/TEDGM, but before the receptor was eluted with the 0.2 M-KCl/TEDGM wash.

To prepare activated receptor, cytosol was prepared as described above, except that the homogenization occurred in 2 vol. of TEDG. The cytosol was then activated by warming at 25 °C for 30 min. DEAE-cellulose-purified activated receptor was prepared by placing activated cytosol on an 8 ml DEAE-cellulose column and washing with 20 ml of TEDG, followed by 20 ml of 0.2 M-KCl/TEDG. The activated 4 S receptor was eluted in the salt wash and dialysed against TEDG to remove the KCl.

Activation-enhancer assay

To test for activation-enhancing activity, the receptor was incubated with either the fraction to be tested or buffer only at 0–4 °C or 25 °C for 30 min, as indicated in the Figure legends. The mixture was then cooled to 0–4 °C, and efficacy of activation was monitored by DNA-cellulose-binding ability.

DNA-cellulose-binding assay

DNA-cellulose was prepared by the method of Alberts & Herrick (1971) and contained 250 µg of calf thymus DNA/ml (packed volume). All steps were performed at 0–4 °C. At the end of the activation-enhancer assay, the mixture was cooled to 0–4 °C, and 0.4 ml of 50 % (v/v) DNA-cellulose in 20 mM-Tris/HCl (pH 7.5) / 1.5 mM-EDTA/1 mM-dithiothreitol (TED) was added and the incubation was continued on ice for 10 min with frequent mixing. The mixture was then poured into glass columns and the initial eluate was collected. The column was washed with 5 ml of TED. This, together with the initial eluate, constitutes the unbound receptor. The bound receptor was eluted with 4 ml of 0.6 M-KCl/TED. Samples of each fraction were then processed for scintillation counting. The percentage bound was obtained by dividing the radioactivity released in the high-salt wash by the total radioactivity in the combined low- and high-salt wash. All experimental determinations were done in duplicate or triplicate, and the variation for each individual value never exceeded 10 % of the average.

Control experiments show that the quantity of DNA-

cellulose used in these assays has a binding capacity at least 5 times greater than the amount of receptor actually retained. There is also no increase in receptor binding to DNA if the incubation is longer than 10 min (results not shown).

Sucrose-gradient centrifugation

This was performed in a Beckman L8-70M ultracentrifuge with a VTi 65.2 rotor. Samples (0.2–0.3 ml) were layered on to 5–20 % (w/v) sucrose gradients in TEDGM and centrifuged at 65 000 rev/min (372 000 *g* at *r*_{av.} of 8.13 cm) for 2.5 h. After centrifugation, the tubes were punctured at the bottom, and 0.2 ml fractions were collected and processed for scintillation counting. [methyl-¹⁴C]Ovalbumin (3.7 S) and [¹⁴C]aldolase (7.9 S) served as internal sedimentation markers.

Miscellaneous

[¹⁴C]Aldolase was prepared by formylation of aldolase by the procedure of Means & Feeney (1968). Protein concentration was determined by the Bio-Rad protein assay, according to the manufacturer's directions.

RESULTS

In order to prepare 9–10 S unactivated receptor, we chromatographed cytosol prepared in the presence of molybdate on a DEAE-cellulose column. The column was first washed with 0.05 M-KCl in molybdate-containing buffer, and the receptor was eluted with 0.2 M-KCl in molybdate-containing buffer. After removal of molybdate by dialysis, we monitored the ability of the receptor to bind DNA-cellulose. We found substantial variability in the percentage DNA-binding of a given receptor preparation after heat activation, from lows of 5 % to highs of 40 %. Some of this variation is undoubtedly due to activation occurring during dialysis. However, in order to account more clearly for this variability, we reasoned that some factor may be being differentially removed from the receptor during the DEAE-cellulose chromatography. To test this hypothesis, we added various amounts of the low-salt wash to the DEAE-cellulose-purified receptor before heat activation and examined the DNA-binding ability of the receptor. As shown in Fig. 1, the addition of the low-salt wash substantially stimulated the DNA-binding ability of the receptor. In this case, the low-salt-wash factor gave a 4.5-fold stimulation. The actual stimulation varied with the preparation of the receptor and the factor. Indeed, we have preliminary evidence that the variability is due to the receptor preparation. If the DEAE-cellulose column is washed with 10 mM-MgCl₂/TEDGM after the 0.05 M-KCl/TEDGM wash but before the 0.2 M-KCl/TEDGM, the resulting receptor displays lower control DNA binding and is stimulated by 600 % by the factor (C. Richardson, O. Olevsky & J. L. Tymoczko, unpublished work). The nature of this effect requires investigation.

This stimulation by the low-salt wash was also clearly dose-dependent. Inasmuch as it has been reported that steroid receptor can be activated by dilution (Higgins *et al.*, 1973), we needed to establish that the effect of the low-salt wash was not due to dilution effects alone. When receptor was incubated with a volume of buffer equivalent to that of the salt wash, there was some stimulation of DNA binding, but not nearly to the same extent as with

Fig. 1. The low-salt wash of cytosol applied to a DEAE-cellulose column stimulates DNA binding of the glucocorticoid receptor

DEAE-cellulose-purified receptor (52000 d.p.m./0.20 ml) was incubated at 25 °C for 30 min with the indicated amount of low-salt wash of the DEAE-cellulose column or buffer only, and then processed from DNA-cellulose binding as described in the Materials and methods section.

the low-salt wash (Fig. 1), demonstrating that the stimulatory effect is not merely a dilution effect. It is also possible that the additional protein in the DEAE-cellulose low-salt wash is non-specifically stabilizing the receptor, thereby enhancing DNA binding. That this is not the case was evidenced by an inability of ovalbumin or other exogenous proteins to enhance DNA binding (results not shown). We next determined how rapidly the

factor enhanced activation. Factor needed to be present for only the final 2.5 min of the 30 min incubation to stimulate DNA binding by approx. 60% relative to the control, or for the final 10 min of the incubation to be as effective as factor that had been present for the entire 30 min (results not shown).

Our previous results have shown that the DNA-binding ability of the receptor is dependent on an alteration in the sedimentation coefficient, from 9–10 S to smaller values (Tymoczko & Phillips, 1983; Tymoczko *et al.*, 1984). To examine whether the low-salt wash factor was able to alter the sedimentation value of the 9–10 S receptor, we incubated the receptor with the factor or with buffer only. As shown in Fig. 2, the factor altered the sedimentation coefficient from 9–10 S to 4–5 S, relative to the undiluted control. In fact, there were usually two peaks, one at approx. 5 S and a shoulder at 4 S. We had previously reported that RNAase treatment of the 7–8 S receptor generates a 5 S form, whereas high-salt treatment yields the 4 S receptor (Tymoczko *et al.*, 1986). The alteration in *s* value was not due simply to dilution, as shown in Fig. 2. Receptor diluted with buffer displayed some shift in sedimentation profile, but not nearly to the extent of factor-treated receptor. We conclude then that the low-salt wash of the DEAE-cellulose column can enhance DNA-binding ability of the receptor while also shifting the *s* value to 4–5 S. This is consistent with the idea that the slower-sedimenting forms of the receptor are best able to bind to DNA-cellulose.

The fact that the factor(s) stimulates DNA binding may be explained in two ways. The factor could enhance the proportion of receptor that is activated, or enhance the binding ability of activated receptor. To examine which of these possibilities is correct, we incubated the receptor with or without factor at 25 °C for 30 min. The receptor without factor was then mixed with factor, and the incubation of both receptor preparations was continued on ice for 30 min. As shown in Fig. 3, the stimulatory effect on DNA-binding was maximal if the factor was present during the initial incubation. This

Fig. 2. Ability of the low-salt-wash factor to alter the sedimentation coefficient of the glucocorticoid receptor

DEAE-cellulose-purified receptor (30000 d.p.m./0.1 ml) was incubated at 25 °C for 30 min without any addition (a), with 2 vol. of buffer only (b) or with 2 vol. of the low-salt wash (c). After the incubation, the samples were layered on to 5–20% sucrose gradients in TEDGM, centrifuged and processed as described in the Materials and methods section.

Fig. 3. The activation factor must be present during the 25 °C incubation to exert its maximal effect

DEAE-cellulose-purified receptor (55000 d.p.m./0.2 ml) was incubated either alone or with the indicated amounts of factor. At the end of this incubation, factor was added to the receptor that had been incubated alone, and both sets of receptors were then incubated on ice for an additional 30 min before measuring DNA-cellulose binding.

suggests that the factor is acting as an activation enhancer, perhaps leading to a conversion of the receptor into a form with better DNA-binding abilities, such as the 4 S and 5 S forms.

If the factor is acting as an activation enhancer, then its effects should not be as evident with preparations of activated receptor. To test this idea, we examined the effects of the factor on DEAE-cellulose-purified activated receptor and activated unfractionated cytosolic receptor. When increasing amounts of the factor were incubated with the various receptors at 25 °C for 30 min and then assayed for the ability to bind to DNA-cellulose, the stimulation of DNA binding was most evident with the DEAE-cellulose-purified unactivated receptor (Fig. 4). In this experiment, the factor stimulated 9–10 S receptor 2.5-fold more effectively than pre-activated DEAE-cellulose-purified receptor or unfractionated cytosolic receptor. The stimulation observed with activated receptors may be due to the presence of some unactivated receptor in these preparations. Alternatively, even these so-called activated preparations may not have achieved maximal DNA-binding ability by thermal activation alone. These results are consistent with the factor playing the role of activation enhancer. For each receptor preparation, the maximal binding achieved was nearly the same (approx. 50%), suggesting that there may be other factors limiting receptor–DNA interaction. Unlike the case with unactivated 9–10 S receptor, the factor had no effect on the sedimentation value of either the DEAE-cellulose-purified activated receptor, which sedimented at 4 S, or the activated unfractionated cytosolic receptor, which sedimented at 7–8 S (results not shown).

Fig. 4. Ability of the activation factor to stimulate DNA binding of activated receptor

DEAE-cellulose-purified unactivated receptor (●; 50000 d.p.m./0.2 ml), DEAE-cellulose-purified activated receptor (▲; 40000 d.p.m./0.2 ml) and activated unfractionated cytosol (○; 42000 d.p.m./0.2 ml) were mixed with the indicated volume of factor and incubated at 25 °C for 30 min. Control values (DNA binding in the absence of factor) were: unactivated receptor, 25%; activated receptor, 42%; activated unfractionated cytosol, 40%.

We have previously reported that RNAase can increase the DNA-binding ability of 7–8 S receptor as well as lower its sedimentation value (Tymoczko & Phillips, 1983), and others have reported that RNAase A can act as an activation enhancer for purified 9–10 S receptor (Webb & Litwack, 1986). Under the conditions described here, RNAase A did not stimulate the DNA binding of the DEAE-cellulose-purified unactivated receptor. Moreover, RNAase had no effect on the sedimentation profile of the 9–10 S receptor, nor did the activation enhancer display any ability to degrade tRNA (results not shown). We can conclude that the activation enhancer is not an RNAase A-like activity.

To establish the nature of the activation enhancer, we preincubated it with various hydrolytic enzymes and tested the treated factor's ability to stimulate receptor DNA binding. As shown in Table 1, the factor's activity was insensitive to RNAase treatment and moderately sensitive to chymotrypsin treatment. Pretreatment of the factor with trypsin, however, eliminated its ability to stimulate DNA binding, thus demonstrating that the activation enhancer is a protein. Chromatography of the dialysed low-salt wash on a Bio-Gel A1.5 m column showed that the factor was actively eluted in the region of 70000 Da (results not shown).

Table 1. Sensitivity of the activation factor to various hydrolytic enzymes

The DEAE-cellulose low-salt wash (0.4 ml) containing the activation factor was incubated with 200 μ g of trypsin, chymotrypsin, RNAase A or buffer only for 30 min at 37 °C. Proteolytic activity was stopped by the addition of 10-fold excess of lima-bean trypsin inhibitor, an amount previously shown to be effective at inhibiting the proteinases (Tymoczko & Lee, 1985). After this incubation, the treated factors were mixed with DEAE-cellulose-purified unactivated receptor (43 000 d.p.m./0.2 ml) and incubated for 30 min at 25 °C and then assayed for DNA-cellulose-binding ability. The control is receptor that was incubated with buffer only. Each value represents the average of duplicate or triplicate determinations. Variations for each individual value never exceeded 10 % of the average.

Conditions	Receptor bound (%)	Stimulation (fold)
Control	26	1.0
+ factor	52	2.0
+ RNAase-treated factor	54	2.1
+ chymotrypsin-treated factor	43	1.7
+ trypsin-treated factor	28	1.1

Fig. 5. Effect of ATP on the ability of the activation factor to enhance DNA binding by the receptor

DEAE-cellulose-purified unactivated receptor (49 000 d.p.m./0.2 ml) was incubated with 0.4 ml of the low-salt wash from DEAE-cellulose column or with 0.4 ml of buffer only for the indicated times at 0–4 °C in the absence or presence of 3 mM-ATP (pH adjusted to 7.5). The reaction mixtures were then processed for the DNA-cellulose-binding assay.

There have been several reports that ATP can lead to the activation of the glucocorticoid receptor when the receptor is incubated with the nucleotide at 0 °C (John & Moudgil, 1979; Moudgil & John, 1980). Although the activation enhancer described here was non-diffusible and therefore not likely to be ATP alone, we reasoned that the enhancer may be associated with ATP. In the presence of receptor, this ATP might become available to the receptor. However, when the DEAE-cellulose-purified unactivated receptor was incubated at 0 °C with ATP, there was an inhibition of activation (Fig. 5). This result differs from those obtained by others using unfractionated cytosolic receptor, in which it was shown that ATP stimulates activation of the glucocorticoid receptor from rat liver (Moudgil & John, 1980) or rat thymus (Holbrook *et al.*, 1983). Moreover, if ATP was included in the incubation with the activation enhancer, the ability of the factor to stimulate DNA binding was greatly diminished. It is still not clear whether the ATP is acting directly on the receptor, on the factor, or both. The nature of this ATP effect requires investigation.

DISCUSSION

It is well established that the unactivated glucocorticoid receptor sediments at 9–10 S in molybdate-containing sucrose gradients. Upon activation under low-salt conditions, a 7–8 S receptor is formed. We have shown that this form, although associated with RNA, is nonetheless capable of binding to DNA (Tymoczko *et al.*, 1984). To achieve maximal DNA binding, the receptor must be converted into slower-sedimenting forms. This can be achieved by passing the 7–8 S receptor over a DEAE-cellulose column, which yields the 4 S receptor (Sakaue & Thompson, 1977; Tymoczko & Lee, 1985), or by treating the 7–8 S receptor with RNAase A, yielding an approx. 5 S form (Tymoczko & Phillips, 1984; Rossini, 1985; Tymoczko *et al.*, 1986). Here we have presented evidence that DEAE-cellulose-purified 9–10 S receptor can be converted directly into the slower-sedimenting 4–5 S forms by a factor that is eluted in the low-salt wash when the receptor preparation is chromatographed on DEAE-cellulose. This factor also enhances DNA-binding ability by 50–600 %, depending on the receptor preparation. Trivial effects cannot be used to explain these results, as neither dilution alone nor stabilization by additional protein mimic the effects of this factor. We believe that this factor is acting as an activation enhancer, since its effects are maximal on unactivated receptor and, further, it must be present during the 25 °C incubation to exert its greatest effects. On the basis of its elution profile from Bio-Gel A1.5 m and its sensitivity to trypsin, the factor is a protein of approx. 70 000 Da.

This factor is apparently distinct from one described by Okamoto *et al.* (1984), which stimulated nuclear binding of already activated glucocorticoid receptor in an ATP-dependent fashion. Although this protein is also recovered in the low-salt wash of a DEAE-cellulose chromatograph of a receptor preparation, it differs in several important ways from the activation enhancer that we describe here. It is most effective with activated receptor, whereas the activation enhancer is most active with unactivated receptor. This protein is of 93 000 Da, compared with approx. 70 000 for the factor described

here, and its activity is stimulated by ATP, whereas that of the activation enhancer is inhibited.

There are reports of a factor similar to the one that we describe here which is capable of converting highly purified unactivated 9–10 S glucocorticoid receptor directly into the 4 S form (Schmidt *et al.*, 1985, 1986; Webb & Litwack, 1986). Those workers suggest that the factor is RNAase, because RNAase A can substitute for the factor. In our hands, RNAase A has no effect on the 9–10 S form of the receptor (the present work; Anderson & Tymoczko, 1985). It is not clear why RNAase A has no effect on the partially purified 9–10 S receptor used in these studies while having a dramatic effect on the highly purified 9–10 S receptor. Those workers did note that actual RNA hydrolysis by the RNAase is not required for activation stimulation to occur, but an intact RNA-binding site on the RNAase is (Schmidt *et al.*, 1986).

Although the nature of the activation factor that we describe is still unclear, it is interesting to speculate on its function in light of its similarity to certain other proteins. Others have reported that a similar-sized protein (72000 Da) is associated with the glucocorticoid receptor from rat liver (Carlstedt-Duke & Gustafsson, 1986). These workers suggest that this protein interacts with the receptor on the chymotrypsin-sensitive domain and plays a role in regulating receptor–DNA interactions. This is the location that we have postulated as the site of interaction of RNA (Tymoczko & Lee, 1985), as well as non-steroid-binding protein factors (Tymoczko *et al.*, 1987), with the receptor. We have also suggested that these factors may play a role in regulating receptor function. Conceivably, the 70000-Da protein that we describe here may be loosely associated with the receptor, and dissociates during the DEAE-cellulose purification. In fact, the 72000-Da protein's interaction with the receptor is not very tight (Carlstedt-Duke & Gustafsson, 1986; Gustafsson *et al.*, 1986), so it is quite possible that it would be lost during ion-exchange chromatography. Those workers suggest that this protein is associated with the receptor when it interacts with DNA (Gustafsson *et al.*, 1986). Others have described a similar protein that is associated with activated human progesterone receptor (Estes *et al.*, 1987). We have yet to establish whether the activation factor remains associated with the receptor after enhancing activation. Whether the protein that we describe has any relationship to the 72000-Da protein found in some preparations of receptor will require further characterization of the activation enhancer.

We thank Carol Horan for assistance in the preparation of this manuscript. This work was supported by grant DCB-8602450 from the National Science Foundation.

REFERENCES

- Alberts, B. & Herrick, G. (1971) *Methods Enzymol.* **21**, 198–217
- Anderson, E. E. & Tymoczko, J. L. (1985) *J. Steroid Biochem.* **23**, 299–306
- Beato, M. & Feigelson, P. (1972) *J. Biol. Chem.* **250**, 1080–1086
- Carlstedt-Duke, J. & Gustafsson, J.-A. (1986) in *Thioredoxin and Glutaredoxin Systems: Structure and Function* (Holmgren, A., Bränden, C.-I., Jörnvall, H. & Sjöberg, B.-M., eds.), pp. 391–401, Raven Press, New York
- Estes, P. A., Suba, E. J., Lawler-Heavner, J., Elashry-Stowers, D., Wei, L. L., Toft, D. O., Sullivan, W. P., Horwitz, K. B. & Edwards, D. (1987) *Biochemistry* **26**, 6250–6262
- Gustafsson, J. A., Carlstedt-Duke, J., Okert, S. & Wikstrom, A.-C. (1986) in *The Role of Receptors in Biology and Medicine* (Gotto, A. M. & O'Malley, B. W., eds.), pp. 1–8, Raven Press, New York
- Higgins, S. J., Rousseau, G. G., Baxter, J. D. & Tomkins, G. M. (1973) *J. Biol. Chem.* **248**, 5866–5872
- Holbrook, N. J., Bodwell, J. E. & Munck, A. (1983) *J. Biol. Chem.* **258**, 14885–14894
- John, J. K. & Moudgil, V. K. (1979) *Biochem. Biophys. Res. Commun.* **90**, 1242–1248
- Kovacic-Milivojevic, B. & Vedeckis, W. V. (1986) *Biochemistry* **25**, 8266–8273
- Means, G. E. & Feeney, R. E. (1968) *Biochemistry* **7**, 2192–2201
- Moudgil, V. K. & John, J. K. (1980) *Biochem. J.* **190**, 799–808
- Okamoto, K., Isohashi, F., Horiuchi, M. & Sakamoto, Y. (1984) *Biochem. Biophys. Res. Commun.* **121**, 940–945
- Rossini, G. P. (1985) *J. Steroid Biochem.* **22**, 47–57
- Sakaue, Y. & Thompson, E. B. (1977) *Biochem. Biophys. Res. Commun.* **77**, 533–541
- Schmidt, T. J., Miller-Diener, A., Webb, M. L. & Litwack, G. (1985) *J. Biol. Chem.* **260**, 16255–16262
- Schmidt, T. J., Diehl, E. E., Davidson, C. J., Puk, M. J., Webb, M. L. & Litwack, G. (1986) *Biochemistry* **25**, 5955–5966
- Tymoczko, J. L. & Lee, J. H. (1985) *Biochim. Biophys. Acta* **846**, 193–199
- Tymoczko, J. L. & Phillips, M. M. (1983) *Endocrinology* (Baltimore) **112**, 142–149
- Tymoczko, J. L., Phillips, M. M. & Vernon, S. M. (1984) *Arch. Biochem. Biophys.* **230**, 345–354
- Tymoczko, J. L., Anderson, E. E., Lee, J. H. & Unger, A. L. (1986) *Biochim. Biophys. Acta* **888**, 296–305
- Tymoczko, J. L., Anderson, E. E., Lee, K. A. & Unger, A. L. (1987) *Biochim. Biophys. Acta* **930**, 114–121
- Unger, A. L., Uppaluri, R., Ahern, S. & Tymoczko, J. (1987) *J. Cell Biol.* **105**, Abstr. 36
- Webb, M. L. & Litwack, G. (1986) *Biochem. Actions Horm.* **13**, 379–402
- Webb, M. L., Schmidt, T. J., Robertson, N. M. & Litwack, G. (1986) *Biochem. Biophys. Res. Commun.* **140**, 204–211