Steroid hormones and temperature induce changes of binding parameters of their receptors in intact cells

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Abstract When MCF-7 cells were treated with 17β-estradiol, dexamethasone, or promegestone at 37°C, the K_D of receptors for their cognate ligands was found to decrease as compared to that measured at 2°C. Cell incubation with hormone at 37°C did not affect the B_{max} of glucocorticoid and progesterone receptors, but caused a 40% increase of that of estrogen receptor. This increase required the presence of ligand, was insensitive to cycloheximide, and was completed within 10 min of cell incubation at physiological temperature. We conclude that an early step of estrogen action is the recruitment of pre-existing receptor molecules through activation of their binding capacity.

Key words: Hormone receptor; Binding capacity; Steroid hormone action; Estrogen; Progesterone; Glucocorticoid

1. Introduction

Steroid hormones trigger physiological responses by binding to their cognate receptors in target cells [1]. The formation of steroid–receptor complexes is considered a key step controlling the functional properties of receptor proteins and their capacity to modulate the expression of specific genes in target tissue [1], so that the mechanisms controlling the cellular levels of receptors have been extensively investigated. One intriguing conclusion which has been obtained in several studies is that steroid receptors can exist in forms unable to bind their cognate ligand [2–11], and that steroid binding capacity of receptors can go through cycles of inactivation–reactivation in intact cells [3–6,12–14].

In a previous investigation we found that the binding capacity of androgen receptor (AR) is post-translationally up-regulated by the ligand in MCF-7 cells [11]. We then thought it was important to extend this study to other steroid receptors expressed in the same cells, and in this report we show that steroid hormones induce an increase in the B_{max} of estrogen (ER) but not glucocorticoid (GR) or progesterone (PR) receptors under physiological conditions.

2. Experimental

2.1. Materials

5α-Dihydro[1,2,4,5,6,7-^{3}H(N)]testosterone (118.5 Ci/mmol), 17β-[2,4,6,7-^{3}H(N)]estradiol (104 Ci/mmol), [6,7-^{3}H(N)]dexamethasone (44 Ci/mmol), and [17α-methyl-^{3}H]promegestone (R-5020; 86 Ci/mmol) were purchased from DuPont NEN. All other reagents were of analytical grade.

2.2. Cell culture conditions

MCF-7 cells were grown in Petri dishes, as previously described [11]. Four days before the experiment, cells were seeded in the culture medium lacking phenol red and containing 10% charcoal-stripped [15] foetal calf serum. On the day of the experiment, cells were harvested with trypsin, and were washed twice by suspension in 20 mM phosphate buffer, pH 7.4, 0.15 M NaCl (PBS) and centrifugation for 8 min at 600 ×g.

2.3. Measurement of binding capacity of steroid hormone receptors in MCF-7 cells

Cells were dispersed in Dulbecco's MEM lacking phenol red and containing 50 mM HEPES buffer, pH 7.3. Cell suspensions were then incubated as specified in the text with the indicated concentrations of tritiated ligand and in the presence or absence of a 200-fold molar excess of nonradioactive ligand as competitor. When binding capacity of PR was measured, the incubation medium included 1 μM nonradioactive cortisol, to prevent R-5020 binding to GR [16]. At the end of the incubations, cells were washed three times with PBS, and were then homogenized with a Dounce homogenizer, after a 30 min swelling at 2°C in 1 ml of 20 mM Tris-HCl, pH 7.5 at 2°C, 1.5 mM $MgCl_2$, 10 mM NaCl. The homogenates were then brought to a final 0.6 M NaCl concentration, and cellular hormone–receptor complexes were extracted by incubation for 45 min at 2°C, and centrifugation for 30 min at 16,000 × g. The supernatants of this centrifugation were then treated for 10 min at 2°C with a dextran-coated charcoal pellet [17] to remove free steroid, and aliquots were taken for measurements of bound radioactivity. Specific ligand binding to hormone receptors was determined by subtraction of binding obtained in the presence of competitor from that found in its absence.

2.4. Sucrose density gradient analysis of steroid–receptor complexes

Linear 5–20% (w/v) sucrose gradients in a solution composed of 20 mM Tris-HCl, pH 7.5 at 2°C, 1.5 mM EDTA, 5% (v/v) glycerol, 0.3 M NaCl, were employed to analyze steroid–receptor complexes. Cell extracts were layered on the top of the gradients, and centrifugations were performed with a Beckman SW-60 rotor in a Beckman L8-80 ultracentrifuge for 18 h at 50,000 rpm, as previously described [17].

3. Results and discussion

MCF-7 cells represent a useful experimental system to study different steroid hormone receptors in a single cell type, as they express ER, PR, GR and AR [16].

Our initial experiments were thus performed to evaluate whether the levels of binding capacity of those receptors varied when cells are incubated at 2 and 37°C with their cognate ligand. By measurement of the specific binding sedimenting at about 4 S in sucrose density gradients (Fig. 1), it was found that the levels of steroid–receptor complexes measured after cell incubation at 37°C are higher than those found if cells are maintained at 2°C. As already established in the case of AR [11], the temperature-dependent increase in binding capacity of ER, GR and PR did not depend on new receptor synthesis, as cell incubation with cycloheximide did not prevent the phenomenon (Fig. 1). Furthermore, these observations were not due to uncomplete attainment of the equilibrium in the binding reaction at 2°C, as steady-state levels of steroid–receptor complexes were already reached within 60 min of incubation at low tem-

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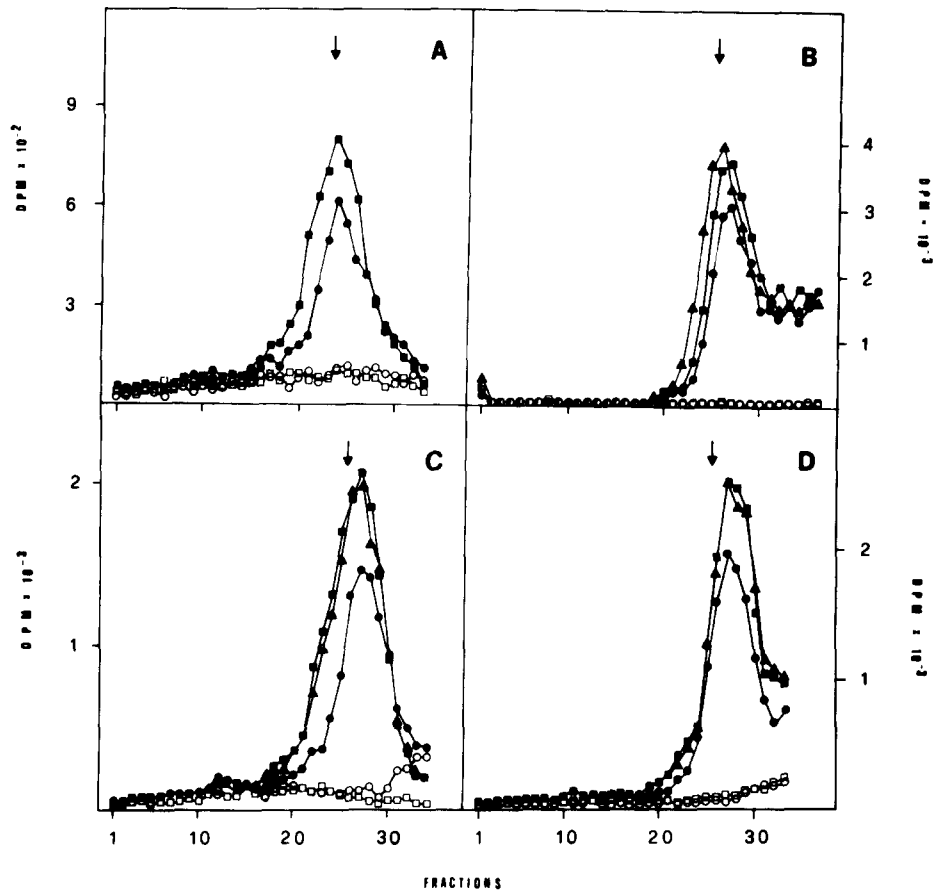

Fig. 1. Analysis of AR, PR, ER and GR from MCF-7 cells by sucrose density gradient centrifugation. Analysis of AR (panel A), PR (panel B), ER (panel C), and GR (panel D) were performed by incubation of MCF-7 cells with tritiated 5α-dihydrotestosterone (5 nM), R-5020 (10 nM), 17β-estradiol (5 nM), or dexamethasone (50 nM), respectively, and in the presence (open symbols) or in the absence (closed symbols) of a 200-fold molar excess of nonradioactive ligand. The incubation medium also contained (▲) or not (○, ●, □, ■) 1 mM cycloheximide. Cells were incubated for 90 min at 2°C followed by 30 min at either 2 (○, ●) or 37°C (□, ■, ▲). At the end of the incubation, cells were processed to prepare extracts, which were subjected to sucrose density gradient centrifugation. The sedimenting position of bovine serum albumin (4.4 S) is indicated by an arrow.

perature (not shown). When the time-course of the phenomenon was evaluated at physiological temperature, instead, we found that upon shifting the temperature of cell incubation from 2 to 37°C, new steady-state levels of steroid hormone binding were obtained within 10 min (Fig. 2).

We then determined the binding parameters of ER, GR and PR (Table 1), and found that MCF-7 cell incubation with hormone at 37°C led to a decrease (about three-fold) of the K_D for their ligand. Interestingly, a temperature-dependent increase of the B_{max} measured for these receptors could be consistently found only in the case of ER. Thus, the highest level of glucocorticoid and progestin binding observed at 37°C in determinations performed with a single hormone concentration (Figs. 1 and 2) could be explained by an increase of affinity of receptors for their cognate ligand. In the case of ER, instead, new binding sites were actually unmasked upon cell incubation with hormone at physiological temperature. This increase of estrogen binding sites depended on both ligand and temperature. When MCF-7 cells were treated for 30 min at 37°C in the absence of ligand and were then incubated with 17β-estradiol at 2°C, in fact, binding levels detected in soluble extracts essentially coincided with those measured in extracts from untreated cells (Table 2).

These findings complement those obtained with AR [11], and support the conclusion that steroid hormones induce post-translational changes of binding parameters of their cognate receptor proteins. Our data show that the phenomenon involves activation of binding capacity of pre-existing inactive

Table 1
Determination of binding parameters of ER, GR, and PR in MCF-7 cells at 2 and 37°C

	B_{max} (pmol/mg DNA)		K_D ($\times 10^{-9}$ M)	
	2°C	37°C	2°C	37°C
ER	2.1 ± 0.2	2.9 ± 0.4	1.2 ± 0.5	0.5 ± 0.1
GR	1.2 ± 0.2	1.3 ± 0.2	20.3 ± 3.8	8.5 ± 3.1
PR	4.9 ± 1.6	4.8 ± 1.3	12.9 ± 2.3	3.8 ± 0.6

Measurements were performed by incubation of cell suspensions with increasing concentrations of tritiated 17β-estradiol (ER), dexamethasone (GR), or R-5020 (PR), either in the presence or in the absence of a 200-fold molar excess of nonradioactive ligand as competitor. In the case of PR measurements, all samples also received 1 μM nonradioactive cortisol. Cells were incubated for 90 min at 2°C, followed by treatment of paired samples for 30 min at either 2 or 37°C. Specific binding was determined, and data were subjected to Scatchard transformation [18]. Values represent means ± S.D. of data obtained from three separate experiments.

receptor molecules in the case of ER (Fig. 1 and Table 1) and AR [11], whereas this could not be detected with GR and PR. As several groups have reported the existence of inactive forms of both GR and PR [3–5,7,9], and it has been also established that binding capacity of steroid hormone receptors follows rapid cycles of inactivation/reactivation in intact cells [3,5–7], we cannot exclude that activation of GR and PR might be compensated and masked by inactivation under our experimental conditions.

While the hormone-dependent regulation of binding capacity of GR and PR in MCF-7 cells remains to be clarified, the results we obtained regarding AR [11], GR, PR, and ER (this paper) in a single cell line, indicate the existence of specific patterns of control of binding parameters of these receptors. Indeed, the existence of some form of selectivity among different hormone and receptor systems is indicated by our findings that the binding capacity of ER measured at 2°C was unaffected after MCF-7 cells had been pretreated with the natural androgen. 5α-dihydrotesterone for 30 min at 37°C, and the same results was obtained when this type of experiment was carried out measuring the binding capacity of AR after cell treatment with cortisol (Table 3). As the phenomenon we have observed depends on both ligand and temperature, and displays selectivity among steroid receptor systems, it can be concluded that its control involves hormonal recognition by target cells.

The molecular mechanism(s) responsible for such selective recognition of hormonal signal and fast cellular response is (are) presently unknown. If classic receptors are involved, hormone binding would stimulate some 'activator' of receptors themselves. Evidence that this can indeed occur has been obtained with ER under cell-free conditions, when the reactivating reaction has been shown to consist of phosphorylation of receptor proteins [19–21]. Although it is known that hormone-induced phosphorylation of steroid receptors is involved in eliciting cellular responses, the reaction has been related to optimal modulation of gene transcription, rather than to activation of binding capacity of preexisting receptor molecules [1].

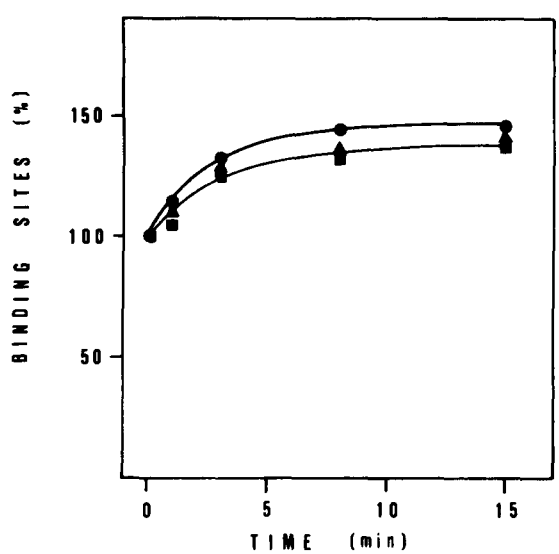

Fig. 2. Time-course of ligand binding to ER, GR, and PR in MCF-7 cells upon a temperature shift from 2 to 37°C. Cell suspensions were preincubated for 90 min at 2°C with 5 nM tritiated 17β-estradiol (5 nM), R-5023 (10 nM), or dexamethasone (50 nM), either in the presence or in the absence of a 200-fold molar excess of nonradioactive competitor. At the end of the preincubation, samples were incubated at 37°C, and at the indicated times aliquots were taken and processed to determine specific ligand binding to ER (●), PR (▲), and GR (■), which has been expressed as the percentage of that found in paired cell suspensions maintained for 2 h at 2°C.

Table 2
Effect of ligand and temperature on binding capacity of ER in MCF-7 cells

Treatment at 37°C	ER binding
None	100
After 17β-estradiol addition	155.5 ± 13.8
Before 17β-estradiol addition	98.0 ± 5.9

MCF-7 cells were preincubated for 30 min at either 2 or 37°C, were brought to 2°C, and were then incubated for 90 min at 2°C with 5 nM tritiated 17β-estradiol either in the presence or in the absence of a 200-fold molar excess of nonradioactive competitor. At the end of the incubation, samples which had been preincubated at 2°C were further incubated for 30 min at 37°C, and those pretreated at 37°C were maintained at 2°C. Paired control samples were maintained at 2°C both before and after ligand addition. The specific binding has been expressed as the percentage of that measured in control samples, and represent means ± S.D. of data obtained from four separate experiments.

Table 3
Effect of MCF-7 cell treatment with steroid hormones on binding capacity of heterologous steroid hormone receptors

Hormone treatment	ER	AR
None	100	100
5α-Dihydrotestosterone	105.2 ± 3.4	N.D.
Cortisol	N.D.	105.7 ± 2.6

MCF-7 cells were treated for 30 min at 37°C with nonradioactive hormone, either 5 nM 5α-dihydrotestosterone or 50 nM cortisol, or without any addition. Samples were then brought to 2°C, and specific binding to ER and AR was determined by cell incubation for 2 h at 2°C with 5 nM tritiated 17β-estradiol or 5α-dihydrotestosterone, respectively, and in the presence or in the absence of a 200-fold molar excess of nonradioactive competitor. The specific binding has been expressed as the percentage of that measured in samples which were pretreated at 37°C without any addition, and values represent means ± S.D. of data obtained from four separate experiments. N.D., not determined.

If hormone recognition and stimulation of the supposed activator involve some other type of receptorial component(s), the phenomenon we have detected would represent a nonclassical hormone action. This interpretation should be considered because the hormone-dependent up-regulation of binding capacity of AR and ER we have observed, displays several features of nonclassical steroid hormone action [22], namely: the effect takes place within few minutes, is not blocked by inhibition of protein synthesis, and is specific with regard to hormone and molecular target (the classical steroid receptor systems). Questions should then be raised regarding the characteristics of the component(s) playing the receptorial role in eliciting this action. While the existence of plasma membrane receptors for steroid hormones has been already reported [22], including the case of ER in MCF-7 cells [23], our study cannot provide insights regarding the involvement of membrane-bound receptors in the phenomenon we have described, because the extracts we have evaluated for hormone binding included only soluble steroid hormone receptors (Fig. 1).

Taken together, our data show that the earliest steps of steroid hormone action involve a hormone-induced change of

binding parameters of classical receptors in target cells, leading to increased affinity of receptors for their cognate ligand and, at least in the case of AR and ER, recruitment of preexisting receptor molecules through activation of their binding capacity. Thus, this phenomenon could be related to the control of optimal responsive potential to hormonal stimuli in target cells [14].

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References

[1] Tsai, M.-J. and O'Malley, B.W. (1994) Annu. Rev. Biochem. 63, 451–486.
[2] Sarff, M. and Gorski, J. (1971) Biochemistry 10, 2557–2563.
[3] Munck, A., Wira, C., Young, D.A., Mosher, K.M., Hallahan, C. and Bell, P.A. (1972) J. Steroid Biochem. 3, 567–578.
[4] Sando, J.J., La Forest, A.C. and Pratt, W.B. (1979) J. Biol. Chem. 254, 4772–4778.
[5] Wheeler, R.H., Leach, K.L., La Forest, A.C., O'Toole, T.E., Wagner, R. and Pratt, W.B. (1981) J. Biol. Chem. 256, 434–441.
[6] Rossini, G.P. and Liao, S. (1982) Biochem. J. 208, 383–392.
[7] Mockus, M.B. and Horwitz, K.B. (1983) J. Biol. Chem. 258, 4778–4783.
[8] Mendel, D.B., Bodwell, J.E. and Munck, A. (1986) Nature 324, 478–480.
[9] Mullick, A. and Katzenellenbogen, B.S. (1986) J. Biol. Chem. 261, 13236–13243.
[10] Auricchio, F. (1989) J. Steroid Biochem. 32, 613–622.
[11] Rossini, G.P. (1991) Biochem. Biophys. Res. Commun. 181, 383–388.
[12] Ishii, D.N., Pratt, W.B. and Aronow, L. (1972) Biochemistry 11, 3896–3904.
[13] Kassis, J.A. and Gorski, J. (1983) Mol. Cell. Biochem. 52, 27–36.
[14] Rossini, G.P. (1984) J. Theor. Biol. 108, 39–53.
[15] Horwitz, K.B. and McGuire, W.L. (1980) J. Biol. Chem. 255, 9699–9705.
[16] Horwitz, K.B., Costlow, M.E. and McGuire, W.L. (1975) Steroids 26, 785–795.
[17] Rossini, G.P. (1985) J. Steroid Biochem. 22, 47–56.
[18] Scatchard, G. (1949) Ann. NY Acad. Sci. 51, 660–672.
[19] Migliaccio, A., Rotondi, A. and Auricchio, F. (1984) Proc. Natl. Acad. Sci. USA 81, 5921–5925.
[20] Raymoure, W.J., McNaught, R.W. and Smith, R.G. (1985) Nature 314, 745–747.
[21] Auricchio, F., Migliaccio, A., Di Domenico, M. and Nola, E. (1987) EMBO J. 6, 2923–2929.
[22] Brann, D.W., Hendry, L.B. and Mahesh, V.B. (1995) J. Steroid Biochem. Mol. Biol. 52, 113–133.
[23] Zänker, K.S., Prokscha, G.W. and Blümel, G. (1981) J. Cancer Res. Clin. 100, 135–148.