MOLECULAR AND CELLULAR BIOLOGY, Dec. 1989, p. 5324–5330
0270-7306/89/125324-07$02.00/0

Vol. 9, No. 12

Different Regions of the Estrogen Receptor Are Required for Synergistic Action with the Glucocorticoid and Progesterone Receptors

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Received 23 May 1989/Accepted 22 August 1989

Estrogen and progesterone or estrogen and glucocorticoid receptors functionally cooperate in gene activation if their cognate binding sites are close to one another. These interactions have been described as synergism of action of the steroid receptors. The mechanism by which synergism is achieved is not clear, although protein-protein interaction of the receptors is one of the favorite models. In transfection experiments with receptor expression vectors and a reporter gene containing estrogen and progesterone-glucocorticoid receptor binding sites, we have examined the effects that different portions of the various receptors have on synergism. N-terminal domains of the chicken progesterone and human glucocorticoid receptors, when deleted, abolished the synergistic action of these receptors with the estrogen receptor. Deletion of the carboxy-terminal amino acids 341 to 595 of the estrogen receptor produced a mutant receptor that could not *trans*-activate on its own. This mutant receptor did not affect the action of the glucocorticoid receptor but functioned synergistically with the progesterone receptor. We therefore conclude that the synergistic action of the receptors for estrogen and progesterone is mechanistically different from the synergistic action of the receptors for estrogen and glucocorticoid.

Steroid hormones modulate gene expression by first binding to distinct receptors in target tissues. The steroid hormone receptor complexes then interact with short discrete nucleotide sequences near or sometimes at considerable distances from the promoters of regulatable genes to modulate their expression (for reviews, see references 4 and 12).

Short palindromic sequences (23, 24, 26, 38) have been shown to mediate glucocorticoid, progestin, and estrogen responses (22–25, 29, 36). In some cases, multiple copies of these sequences mediate the hormone response through the concerted action of the individual receptor binding sites (8–10). In experiments in which chimeric constructs consisting of two or more receptor binding sites were placed in front of a hormone-insensitive promoter, the functional activity of the receptor binding sites was determined to be more than the additive responses of the individual elements (2, 7, 22, 37). This effect was described as synergism of steroid hormone action. Steroid hormone receptor binding sites when placed near the binding sites of other transcription factors also functioned synergistically with these binding sites (35, 37). How functional synergism is achieved is not clear, although protein-protein interactions of the receptors are among the many suggestions put forward to explain this phenomenon (7).

The estrogen, glucocorticoid, and progesterone receptors belong to a large superfamily of ligand-dependent transcription factors (12, 16). These receptors possess modular structures composed of a cysteine-rich DNA-binding domain in the middle portion of the receptors, a carboxy-terminal hormone-binding domain, and an amino-terminal transcription modulator domain. The amino-terminal ends of the various receptors vary extensively in content and size. Nevertheless, the functions of the various domains of the steroid hormone receptors have striking similarities. In the glucocorticoid and progesterone receptors, portions of the N-terminal domains have been shown to possess *trans*-activation function (13, 14, 18, 21). Apart from hormone binding, the carboxy-terminal domains of the different receptors have other functions. They are required for dimerization of the estrogen receptor (26), for hormone-dependent nuclear translocation (34), and for transcriptional inhibition by the glucocorticoid receptor (32). Furthermore, the carboxy-terminal regions in the estrogen, progesterone, and glucocorticoid receptors contain part of the *trans*-activation functions of these receptors (5, 11, 20, 40). A portion of the glucocorticoid and estrogen receptors that extends between the DNA- and hormone-binding domains has been shown to be involved in negative regulation of transcription, possibly through interaction with cell-specific factors (1). Both the carboxy- and amino-terminal regions of the estrogen receptor inhibit progesterone and glucocorticoid responses, and both the progesterone and glucocorticoid receptors inhibit activation by the estrogen receptor (31). These negative actions of the steroid receptors have been suggested to involve competition of the receptors for functionally limiting transcription factors (31). So far, domains of steroid hormone receptors that participate in the synergism of action of steroid hormones have not been defined.

In this study, we examined the regions of the progesterone, glucocorticoid, and estrogen receptors involved in the synergistic action of these receptors described previously by Cato et al. (7) and Ankenbauer et al. (2). These studies were carried out by transient transfection of expression vectors containing wild-type and mutated receptor cDNAs and a chimeric construct containing the cognate binding sites for these receptors into monkey CVI cells. As CVI cells do not contain any functional receptors, the response of the chimeric construct to the transfected receptors could be directly related to the function of the receptors. In this paper,

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we demonstrate that whereas N-terminal amino acid sequences of the progesterone and glucocorticoid receptors are required for synergism, the regions of the estrogen receptor needed for synergistic action with the progesterone receptor are not the same as those needed for synergism with the glucocorticoid receptor. Thus, the synergistic actions of the estrogen-progesterone or estrogen-glucocorticoid receptors are mechanistically different.

MATERIALS AND METHODS

Plasmid DNA construction. Constructions of HEO, previously called $pKCR_2$-pOR8, HE19, and the vector $pKCR_2$ have been described (6, 17, 28). Constructions of cPR1, cPR2, and cPR3 have also been described (18). The chimeric receptor AB(GR)cPR was constructed by insertion of a *Kpn*I site at the DNA sequences that correspond to amino acid 421 of the chicken progesterone receptor and exchanging the N-terminal region of this receptor with the N-terminal domain of the glucocorticoid receptor. Construction of the human glucocorticoid receptor expression vector pRShGRα has been described (13). The expression vector alone without the glucocorticoid receptor cDNA sequences ($pRShR^-$) was constructed from pRShMR (3) by elimination of the *Acc*I-*Bam*HI fragment, followed by a filling-in reaction with DNA polymerase I and blunt-end ligation of the vector sequence. The mutant glucocorticoid receptor phGR9 was constructed from pRShGRα by digestion with *Bgl*II to eliminate the DNA sequences coding for amino acids 77 to 262. The rest of the plasmid was ligated to generate a mutant glucocorticoid receptor with the same reading frame in the amino-terminal and carboxy-terminal ends of the glucocorticoid cDNA. Plasmid p23/4 was constructed by cleaving plasmids $pERE^-PRE^+$ (7) and $pERE^+PRE^-$ (7) with *Hin*dIII and *Bam*HI, respectively, followed by a filling-in reaction with DNA polymerase I to generate blunt ends. Both plasmids were then cleaved with *Bgl*II to generate 207- and 170-base-pair (bp) fragments. The 207-bp fragment from $pERE^-PRE^+$ was cloned in the position of the 170-bp fragment of $pERE^+PRE^-$. The resulting plasmid, p23/4, was then sequenced by the technique of Maxam and Gilbert (30). pE^+P^+OVEC2 was constructed by replacing the *Sac*I-*Pst*I fragment of plasmid OVEC-1 (41) with the *Hin*dIII-*Pst*I fragment of plasmid p23/4. The *Sac*I and *Hin*dIII sites of OVEC-1 and p23/4 were previously filled in with DNA polymerase 1 to generate blunt ends.

Cell culture and transient transfection. Monkey kidney CVI cells were routinely cultured in Dulbecco modified Eagle medium supplement with 10% fetal calf serum. Two days before transfection, the cells were washed once with phosphate-buffered saline and cultured in Dulbecco medium without phenol red (GIBCO Laboratories and Bethesda Research Laboratories, Inc.) but supplemented with 10% fetal calf serum (stripped of hormone by charcoal treatment [42]). The cells were transfected by the calcium phosphate precipitation technique (33). Unless otherwise stated, a typical transfection experiment contained 14 μg of plasmid DNA, of which 10 μg was p23/4 DNA, 1 μg of estrogen, progesterone, or glucocorticoid receptor expression vector, and 2 μg of pCH110, an internal control plasmid coding for β-galactosidase activity (19). The rest of the transfected DNA was made to the 14 μg of total DNA by the expression vector $pKCR_2$ or $pRShR^-$. These vectors also substituted for the receptor expression vectors when no receptor was required in the transfected cells. After transfection, the cells were treated with the following concentration of hormone: 10^{-7} M dexamethasone (Dex), 10^{-7} M 17β-estradiol, or 10^{-8} M R5020.

β-Galactosidase activity and CAT assays. After transfection, the cells were disrupted by repeated freezing in a dry-ice ethanol bath and thawing at 37°C (five times). Cellular extracts were used for β-galactosidase activity as described by Herbomel et al. (19). Chloramphenicol acetyltransferase (CAT) assays were performed as described by Gorman et al. (15) with cellular extracts that contained 6 U of β-galactosidase activity.

S1 nuclease mapping analysis. Analysis of the start of transcription was carried out by using S1 nuclease mapping technique with total RNA isolated from CVI cells cotransfected with the constructs pE^+P^+OVEC2 and OVEC-REF (41) (an internal control plasmid on which the simian virus 40 enhancer increases transcription at the β-globin promoter) and the estrogen and progesterone receptor expression vectors HEO and cPR1 (17, 18, 28). After transfection and treatment of the transfected cells with the various hormones, 35 μg of total RNA was extracted and hybridized with an end-labeled 500-bp *Eco*RI-*Bam*HI fragment of pE^+P^+ OVEC2 labeled at the 5′ end of the *Bam*HI site. The RNA-DNA hybrid was then treated with S1 nuclease by the procedure of Weaver and Weissmann (39).

RESULTS

Synergistic action of progesterone and estrogen was demonstrated in CVI cells by cotransfection of the receptors for these steroids (cPR1 and HEO) and an indicator gene that contains estrogen and progesterone receptor binding sites in close proximity to one another. The receptor binding sites of the indicator gene p23/4 (Fig. 1A) were linked to the thymidine kinase (TK) promoter driving transcription of the bacterial CAT gene. In the transfection experiments, 17β-estradiol (E_2) induced CAT activity from 1.3 to 19.6, whereas the progestin R5020 induced CAT activity to 8.2. Combined treatment of the transfected cells with E_2 and R5020 increased CAT activity to 45.8 (HEO plus cPR1; Table 1), a level higher than the additive effects of E_2 and R5020.

The actions of these steroids reflect correct transcription at the TK promoter, as demonstrated by S1 nuclease mapping experiments. In these studies, the hormone receptor binding elements and the TK promoter of construct p23/4 were isolated and substituted for the β-globin promoter in the construct OVEC-1 (41) to generate the construct pE^+P^+OVEC2 (Fig. 1B). This new construct, together with a control vector, OVEC-REF (41), that contains the simian virus 40 enhancer regulating transcription at the β-globin promoter (Fig. 1C), was cotransfected with the estrogen and progesterone receptors (HEO and cPR1) into CVI cells. After treatment of the transfected cells with hormones, RNAs were extracted and analyzed for transcription at the TK promoter, using the S1 nuclease mapping procedure (39). An S1 nuclease probe generated from pE^+P^+OVEC2 could distinguish between transcripts generated at the TK promoter of pE^+P^+OVEC2 (377 nucleotides; Fig. 1B) and transcripts generated at the promoter of OVEC-REF (330 nucleotides; Fig. 1C). In the S1 nuclease mapping experiments, no transcripts were detected at the TK promoter in the absence of the estrogen and progesterone receptors and their corresponding steroids (Fig. 2, lanes 1 and 2). The estrogen receptor in the presence of E_2 induced transcription at the TK promoter (Fig. 2, lane 3). This level of transcription was not affected by the presence of the unliganded

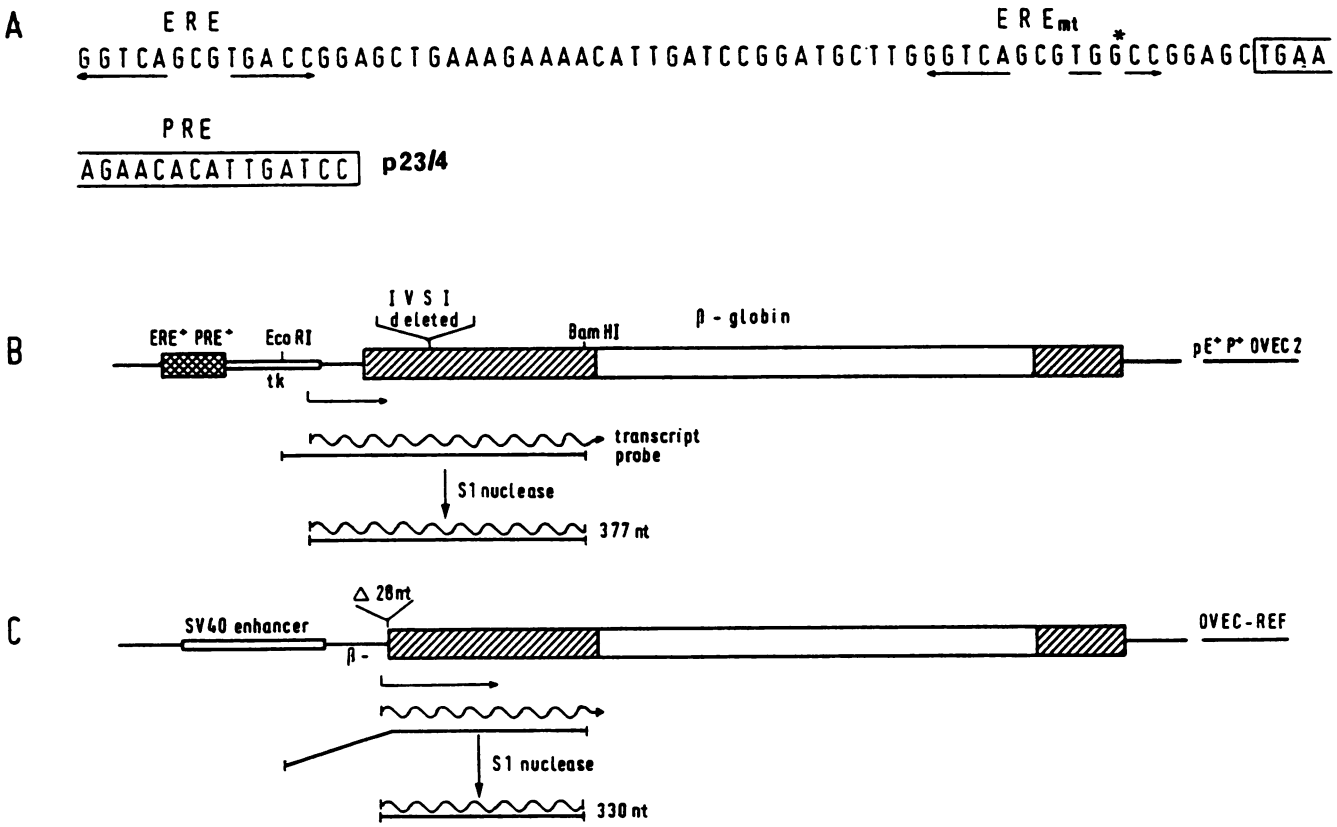

FIG. 1. Schematic representation of the chimeric constructs. (A) Oligonucleotide sequence cloned into the *Hin*dIII and *Bam*HI sites of plasmid pBL-CAT 8^+ (23). Arrows show positions of the perfect and imperfect palindromic estrogen response elements, indicated as ERE and EREmt, respectively. The asterisk indicates the position of a change in nucleotide residue from an adenine into a guanine. This single-base change has been shown to reduce estrogen receptor binding at the estrogen response element (23). (B) Construct pE^+P^+OVEC2. Shown are exon (▨) and intron (□) sequences in the β-globin gene, the *Eco*RI-*Bam*HI fragment used as an S1 nuclease probe, and protected fragments generated after S1 nuclease digestion. Also shown are the TK promoter and the hormone receptor binding sites ERE^+ (estrogen response element) and PRE^+ (progesterone response element. (C) Schematic representation of the construct OVEC-REF (41), in which the simian virus 40 enhancer controls transcription initiated at the β-globin promoter. Indicated is the protected 330-nucleotide S1 nuclease digestion product after hybridization of RNA from the transfected cells with the pE^+P^+OVEC2 S1 nuclease probe.

progesterone receptor (Fig. 2, compare lanes 3 and 4). However, in the presence of the estrogen and progesterone receptors and the hormones E_2 and R5020, a level of transcription much higher than the level in Fig. 2, lane 4, was observed (Fig. 2, compare lanes 4 and 6), although the progesterone receptor alone in the presence of R5020 mediated a level of transcription at the TK promoter that was barely detectable (Fig. 2, lane 5). These results show that the synergistic action of E_2 and R5020 demonstrated with the CAT assay (Table 1) reflects accumulation of the correct transcripts at the TK promoter.

To identify the regions of the progesterone receptor involved in this synergism with the estrogen receptor, different N-terminal deletion mutants of the progesterone receptor (Fig. 3) were combined with the wild-type estrogen receptor HEO in cotransfection experiments with p23/4 in CVI cells.

TABLE 1. CAT activity in CVI cells cotransfected with construct p23/4, the estrogen receptor expression vector HEO, and mutant or chimeric progesterone receptor expression vector F[a]

Hormone	CAT activity[b]				
	HEO + cPR1	HEO + cPR2	HEO + cPR8	HEO + cPR3	HEO + AB(GR)cPR
None (0.07% ethanol)	1.3 ± 0.4	0.7 ± 0.1	0.8 ± 0.3	0.3 ± 0.1	0.6 ± 0.1
E_2	19.6 ± 2.6	14.0 ± 2.8	14.1 ± 2.4	12.7 ± 2.3	23.6 ± 1.1
R5020	8.2 ± 2.8	3.1 ± 0.2	2.8 ± 0.8	0.4 ± 0.1	11.6 ± 1.3
E_2 + R5020	45.8 ± 1.7	17.7 ± 2.3	15.2 ± 1.3	12.0 ± 1.1	62.5 ± 10.1

[a] CVI cells were cotransfected with 10 μg of p23/4, 1 μg of HEO, 2 μg of pCH110, and 1 μg of the progesterone receptor expression vector cPR1, cPR2, cPR8, cPR3, or AB(GR)cPR. After transfection, the cells were treated as indicated.

[b] Determined with cellular extract containing 6 U of β-galactosidase activity and calculated as the percentage of chloramphenicol acetylated per sample of protein extract containing 6 U of β-galactosidase activity. Results are averages and standard deviations of at least five independent experiments using at least two different plasmid preparations. CAT activity and β-galactosidase assays were carried out as described in Materials and Methods.

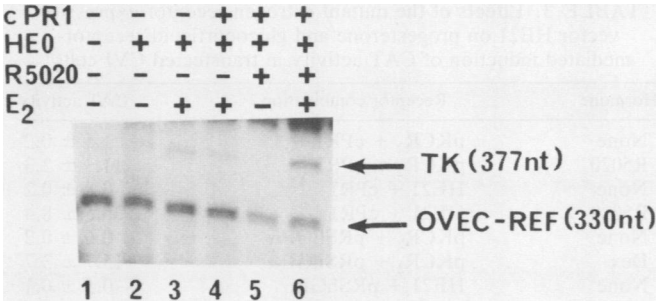

FIG. 2. Products of S1 nuclease mapping of estradiol- and progestin-induced transcription at the TK promoter of transfected pE$^+$P$^+$OVEC2. CVI cells were cotransfected with 14 μg of total plasmid DNA containing 10 μg of pE$^+$P$^+$OVEC2, 2 μg of OVEC-REF, 1 μg of HEO, and 1 μg of cPR1. In transfection experiments carried out without receptor or with only one type of receptor, the vector pKCR$_2$ was used to make up the total amount of DNA transfected. After transfection, the cells were treated with E_2, R5020, or both for 44 h. Thereafter, RNA was extracted and transcription at the TK promoter and of the OVEC-REF construct was determined by the S1 nuclease mapping. The products of S1 nuclease digestion were analyzed on an 8% denaturing gel and viewed by autoradiography. Transcripts initiated at the TK promoter and from the OVEC-REF plasmid are indicated. Shown above the lanes are the combinations of receptor expression vectors transfected and the combinations of hormones administered.

In these experiments, the synergistic action of E_2 and R5020 decreased as the combination HEO plus cPR1 was altered to HEO plus cPR2 (Table 1). This result demonstrates that the N-terminal amino acids 1 to 128 of the progesterone receptor (cPR1 to cPR2) feature prominently in the synergistic action of this receptor with the estrogen receptor. The N-terminal region of the progesterone receptor is also involved in the ligand-dependent *trans*-activation response. The wild-type progesterone receptor cPR1 in the presence of the progestin R5020 induced CAT activity at the TK promoter from 1.3 to 8.2. This value was reduced from 8.2 to 0.4 (Table 1) by deleting N-terminal sequences as in cPR2 and cPR8 (Fig. 3). Mutant cPR3, which has the N-terminal 409 amino acids of the progesterone receptor deleted, in transfection experiments did not show a ligand-dependent *trans*-activation function. The induced activity of 0.4 mediated by cPR3 was not significantly different from the basal activity of 0.3 (HEO plus cPR3; Table 1). This finding is in agreement with the results of Gronemeyer et al. (18) showing that the N-terminal region of the chicken progesterone receptor is required for ligand-induced *trans*-activation. Thus, whereas the entire N terminus of the progesterone receptor is required for the ligand-dependent *trans*-activation function, only the first 128 amino acids of this receptor are required for synergism with the estrogen receptor. In addition, we observed that the basal level of CAT activity was not always identical in the presence of the different N-terminal deletion mutants of the progesterone receptor. The basal activity was reduced from 1.3 to 0.3 as the N-terminal sequences were deleted. This occurred without a significant decrease in the response of HEO (Table 1). The decrease in basal level of expression was most likely a result of transcriptional interference at the TK promoter by the progesterone receptors with deleted N-terminal sequences.

To investigate which sequences on the estrogen receptor interact with the progesterone receptor for synergism, the estrogen receptor mutants HE19 and HE21 (Fig. 3), with N- and C-terminal deletions, were examined in cotransfection experiments with the progesterone receptor wild-type construct cPR1. The mutant receptor HE19 mediated 40% the estrogen response of the wild-type estrogen receptor HEO (Table 2; compare E_2-induced CAT activity of 7.0 with 19.6), a result consistent with the finding that HE19 has a decreased *trans*-activating action on some gene constructs

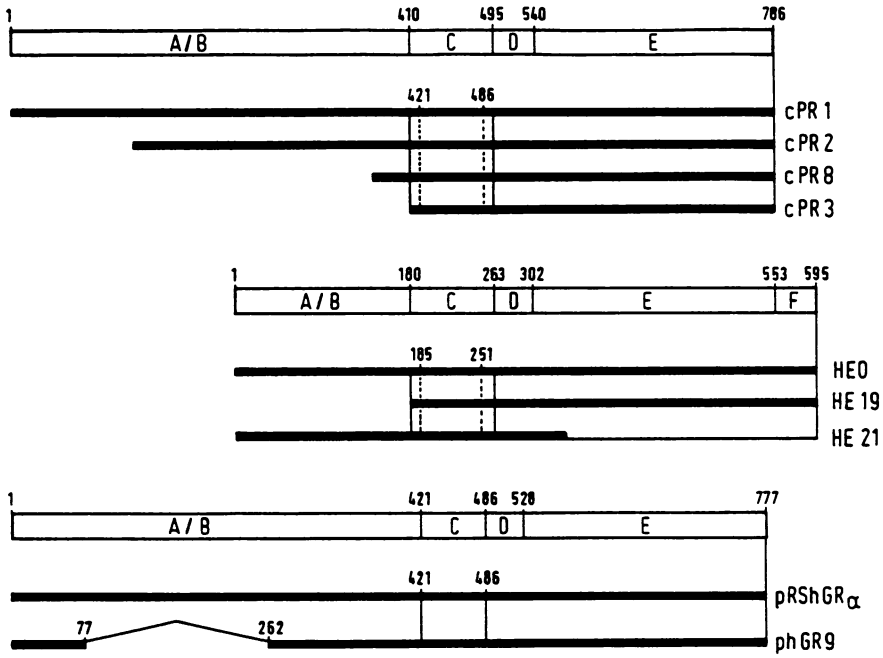

FIG. 3. Schematic diagram of the progesterone, estrogen, and glucocorticoid wild-type and mutant receptors used in the transfection experiments. These receptors have been aligned with reference to their DNA-binding domains (C).

TABLE 2. Effects of N-terminal deletion of the estrogen receptor on the synergistic action of the estrogen and progesterone receptors[a]

Hormone	CAT activity	
	HEO + cPR1	HE19 + cPR1
None (0.07% ethanol)	1.3 ± 0.5	1.5 ± 0.1
E_2	19.6 ± 2.6	7.0 ± 0.9
R5020	8.2 ± 2.8	10.1 ± 0.2
E_2 + R5020	45.8 ± 1.7	37.3 ± 0.8

[a] CVI cells were cotransfected with 10 μg of p23/4, 1 μg of HEO or HE19, 1 μg of cPR1, and 2 μg of pCH110 as described in Materials and Methods. After transfection, the cells were treated as indicated, and CAT activity was determined as for Table 1.

(27). When the transfected cells were treated with E_2 and R5020, CAT activity was induced to a value greater than the additive effect of E_2 and R5020 (Table 2), a result we still considered as synergistic response. Since deletion of the N-terminal sequences of the estrogen receptor in HE19 did not affect its synergistic action with the progesterone receptor, the N-terminal region of the estrogen receptor is not required for this function. In cotransfection experiments with HE21, the C-terminal deletion mutant of the estrogen receptor (Fig. 3), we did not observe any significant *trans*-activating function of this mutant receptor in the presence or absence of E_2 (Fig. 4). This result was due to the deletion in the hormone-binding C-terminal domain of the estrogen receptor and not to unequal amounts of protein synthesized in transfected cells, as shown by Meyer et al. (31). In combination with cPR1, HE21 increased drastically the response of cPR1 (Fig. 4 and Table 3). These results demonstrated that the mutant receptor HE21 still functioned synergistically with the progesterone receptor although it did not trans-activate on its own. The carboxy-terminal region of the estrogen receptor is therefore not required for synergistic action with the progesterone receptor. Thus, the deletions at

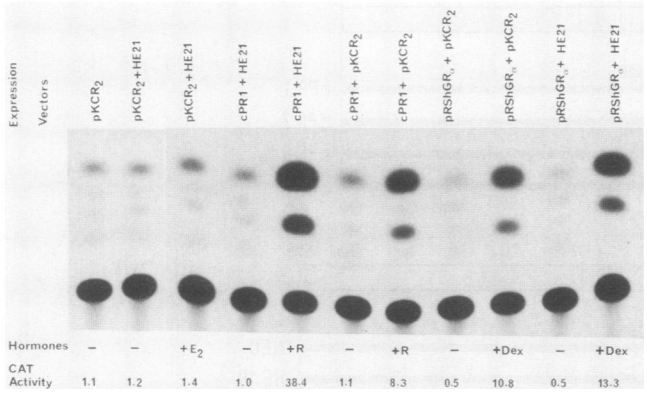

FIG. 4. Effect of the estrogen receptor mutant HE21 on the action of cPR1 and pRShGRα on construct p23/4. CVI cells were transfected with 10 μg of p23/4, 1 μg of vector sequence $pKCR_2$ or HE21, 1 μg of cPR1 or pRShGRα, and 2 μg of pCH110. The transfected cells were treated with 17β-estradiol (E_2), the progestin R5020 (R), or Dex, as indicated. After harvesting, a CAT assay was performed with 6 U of β-galactosidase activity as described in Materials and Methods. The products of the CAT assay were separated by thin-layer chromatography. The combinations of receptor expression vectors used for the transfections are indicated. CAT activity is expressed as percentage of chloramphenicol acetylated.

TABLE 3. Effects of the mutant estrogen receptor expression vector HE21 on progesterone and glucocorticoid receptor-mediated induction of CAT activity in transfected CVI cells[a]

Hormone	Receptor combination	CAT activity
None	$pKCR_2$ + cPR1	1.5 ± 0.2
R5020	$pKCR_2$ + cPR1	11.3 ± 2.3
None	HE21 + cPR1	0.8 ± 0.2
R5020	HE21 + cPR1	36.5 ± 8.4
None	$pKCR_2$ + pRShGRα	0.6 ± 0.2
Dex	$pKCR_2$ + pRShGRα	15.7 ± 3.7
None	HE21 + pRShGRα	0.5 ± 0.1
Dex	HE21 + pRShGRα	12.1 ± 1.2
None	$pKCR_2$ + AB(GR)cPR	1.1 ± 0.1
R5020	$pKCR_2$ + AB(GR)cPR	11.1 ± 2.8
None	HE21 + AB(GR)cPR	1.3 ± 0.2
R5020	HE21 + AB(GR)cPR	11.6 ± 0.2

[a] CVI cells were cotransfected with 10 μg of construct p23/4, 1 μg of HE21 or the vector $pKCR_2$, 1 μg of cPR1, pRShGRα, or AB(GR)cPR, and 2 μg of pCH110. The cells were treated as indicated, and CAT activity was determined as for Table 1.

the N and C termini of the estrogen receptor are individually not required for the synergistic action of the estrogen receptor with the progesterone receptor.

It has been shown that E_2 functions synergistically with the glucocorticoid Dex in mediating CAT activity of a construct similar to p23/4 in cells that contain functional estrogen and glucocorticoid receptors (2). In cotransfection experiments in CVI cells with p23/4 and the expression vectors for the estrogen and glucocorticoid receptors, HEO and pRShGRα (Fig. 3), E_2 and Dex induced CAT activity to a level that was greater than the additive effect of the two steroids (Table 4). Thus, a synergistic action of E_2 and Dex could also be demonstrated with our indicator gene p23/4. Deletion of the N-terminal *trans*-activation domain (τ_1) of the glucocorticoid receptor, which consists of amino acids 77 to 262 (13), resulted in a mutant construct, phGR9 (Fig. 3), with a reduced ability to *trans*-activate (13; Table 4). In CVI cells transfected with p23/4, phGR9, and HEO, treatment with E_2 and Dex did not show the synergistic action of these two steroids (Table 4). Thus, as with the progesterone receptor, N-terminal sequences of the glucocorticoid receptor are involved in synergism with the estrogen receptor. Synergism was also observed with the combination of the N-terminal deleted estrogen receptor and the wild-type glucocorticoid receptor (HE19 plus pRShGRα; Table 4). Thus, the amino-terminal region of the estrogen receptor is not involved in the synergism. This finding is similar to the results we obtained with the estrogen and progesterone receptor combination HE19 plus cPR1 (Table 2). However,

TABLE 4. CAT activity in transfected CVI cells containing wild-type and mutant estrogen and glucocorticoid receptor expression vectors[a]

Hormone	CAT activity		
	HEO + pRShGRα	HEO + phGR9	HE19 + pRShGRα
None (0.07% ethanol)	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
E_2	11.0 ± 2.2	13.7 ± 2.7	6.2 ± 0.9
Dex	7.8 ± 1.6	1.5 ± 0.8	6.1 ± 2.1
E_2 + Dex	41.3 ± 1.0	16.0 ± 1.5	29.0 ± 3.2

[a] CVI cells were cotransfected with 10 μg of p23/4, 1 μg each of the combinations shown, and 2 μg of pCH110. After transfection, the cells were treated as indicated, and CAT activity was determined as for Table 1.

unlike the case with synergistic action of the estrogen and progesterone receptors, the estrogen receptor mutant HE21 was unable to enhance the glucocorticoid response when cotransfected with pRShGRα into CVI cells (Fig. 4 and Table 3; compare Dex response of $pKCR_2$ plus pRShGRα with that of HE21 plus pRShGRα). Thus, the carboxy-terminal sequences 341 to 595 of the estrogen receptor are required for the synergistic action of the estrogen receptor with the glucocorticoid receptor.

To determine whether the N-terminal regions of the glucocorticoid receptor may be responsible for the different behaviors of the glucocorticoid receptor with the estrogen receptor, we studied the response of the construct AB(GR)cPR, in which the N-terminal region of the glucocorticoid receptor replaces the N-terminal region of the progesterone receptor. In cotransfection experiments with p23/4, HEO, and AB(GR)cPR, this chimeric receptor induced CAT activity in the presence of R5020 (Table 1). More important, AB(GR)cPR functioned synergistically with HEO when the transfected cells were treated with E_2 and R5020 (Table 1). Since the expression vector AB(GR)cPR contains the N-terminal domain of the glucocorticoid receptor linked to the progesterone receptor mutant cPR3, which did not respond to R5020 (Table 1; 18), the response of AB(GR)cPR to R5020 must be due to the contribution of the N-terminal region of the glucocorticoid receptor. In cotransfection experiments with AB(GR)cPR and HE21, we found that deletion of the carboxy-end of the estrogen receptor in HE21 abolished the synergistic action of the estrogen receptor with AB(GR)cPR [Table 3; compare the progestin response of $pKCR_2$ plus AB(GR)cPR with that of HE21 plus AB(GR)cPR]. This finding is similar to the result we obtained with pRShGRα and HE21, which led us to the conclusion that HE21 does not function synergistically with the glucocorticoid receptor (Fig. 4 and Table 3). Our results on the lack of synergistic action of HE21 and AB(GR)cPR confirm that the N-terminal region of the glucocorticoid receptor is involved in an interaction with the estrogen receptor that is different from the interaction of the N-terminal region of the progesterone receptor with the estrogen receptor.

DISCUSSION

We have demonstrated in receptor cotransfection studies that the estrogen and progesterone or estrogen and glucocorticoid receptors function synergistically in mediating the response of a chimeric construct that contains estrogen and progesterone-glucocorticoid receptor binding sites. We have determined that the amino acid sequences involved in this synergism are located in the N-terminal regions of the progesterone or glucocorticoid receptors and different regions of the estrogen receptor. Deletion of carboxy- and amino-terminal regions of the estrogen receptor did not affect the synergistic action of the receptor with the progesterone receptor, indicating that a redundant sequence required for the synergistic action with the progesterone receptor is present in both the amino and carboxy termini of the estrogen receptor. Alternatively, sequences encompassing the DNA-binding domain of the estrogen receptor may be required for the synergism. Our results do not distinguish between these two possibilities. In the case of the action of the estrogen and glucocorticoid receptors, whereas deletion of the amino-terminal region of the estrogen receptor had no effect on synergism, deletion of the carboxy-terminal amino acids 341 to 595 of the estrogen receptor abolished the synergistic action of the receptor with the glucocorticoid receptor. The deletion of the carboxy-terminal region of the estrogen receptor generates a mutant receptor that is barely able to *trans*-activate, probably because this mutant is unable to dimerize (26), as dimerization is a prerequisite for functional activity of the estrogen receptor. That this mutant estrogen receptor has no effect on the glucocorticoid response is consistent with a model in which synergism is achieved by the individual functional activity of the various binding components acting on a common effector molecule.

At the N-terminal regions of the progesterone and glucocorticoid receptors, the amino acids that contribute significantly to synergism are amino acids 1 to 128 of the chicken progesterone receptor and amino acids 77 to 262 of the human glucocorticoid receptor. No striking sequence homology has been identified between these two N-terminal sequences except the prevalence of negatively charged amino acid sequences. It remains to be established whether these negatively charged amino acid residues are important for synergism. It has, however, been established that such amino acid residues constitute a property of the *trans*-activation domain of the glucocorticoid receptor (20).

It can be assumed that synergism of steroid hormone action is no more than the potentiation of the *trans*-activating functions of the progesterone and glucocorticoid receptors by the activating function of the estrogen receptor. This description of synergism could hold true for the estrogen and glucocorticoid receptors, but it is incompatible with our results, which clearly show that a mutant estrogen receptor with little or no *trans*-activating property on its own potentiates the action of the progesterone receptor. Such a result is explainable by the implication of some form of direct interaction between the estrogen and progesterone receptors. Alternatively, the mutant estrogen receptor may bind a promoter element and cause a structural change in bound transcriptional complexes that results in no measurable increase in transcription but potentiates the ability of the progesterone receptor to stimulate transcription. In another model, the mutant estrogen receptor and the progesterone receptor could interact indirectly with a common bound protein, which would result in increased affinity of both the mutant estrogen receptor and the progesterone receptor for DNA, leading to an increase in transcription. Whatever the mechanisms involved, our results show that whereas the estrogen and glucocorticoid receptors require each other's *trans*-activation function for their synergistic action to be manifested, this is not the case for the estrogen and progesterone receptors. We therefore propose that the synergistic action of the estrogen and progesterone receptors is achieved through a mechanism different from the synergistic action of estrogen and the glucocorticoid receptors.

ACKNOWLEDGMENTS

We thank S. M. Hollenberg and R. M. Evans for providing the human glucocorticoid receptor expression vector pRShGRα. We are grateful to the Laboratoire de Génétique Moleculaire des Eucaryotes, Strasbourg, France, for providing the wild-type and mutant estrogen and progesterone receptor expression vectors. We thank H. Gronemeyer (Laboratoire de Génétique Moleculaire des Eucaryotes) for helpful suggestions and J. Weinmann for excellent technical assistance.

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