Induction Properties of a Transiently Transfected Glucocorticoid-responsive Gene Vary with Glucocorticoid Receptor Concentration

(Received for publication, July 24, 1996, and in revised form, September 10, 1996)

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Transient transfections of steroid receptors have yielded much of the data used to construct the current models of steroid hormone action. These experiments invariably examine the ability of receptors to regulate transcription when occupied by saturating concentrations of steroid. We now report that other induction properties of a transiently transfected gene are not constant but vary with the concentration of transiently transfected glucocorticoid receptors. Thus, the percentage of maximal induction seen with subsaturating concentrations of glucocorticoid could be dramatically increased, and an antiglucocorticoid could be converted into a partial glucocorticoid, simply by increasing the concentration of glucocorticoid receptors. This behavior was observed in HeLa cells, containing endogenous receptors, or in CV-1 cells, containing almost no endogenous receptor, with either homologous or heterologous receptors. These increases were relatively insensitive to the concentration of reporter gene, suggesting the titration of some transcription factor(s) involved in regulating the position of the glucocorticoid dose-response curve and the agonist activity of an antiglucocorticoid. This property of transfected glucocorticoid receptors required a full-length, functionally active receptor but was retained, albeit reduced in magnitude, in the absence of binding to a glucocorticoid response element. Furthermore, this phenomenon was specific in that the A form of the human progesterone receptor had no effect under the same conditions. These variations in induction properties of antiglucocorticoids and of subsaturating concentrations of glucocorticoid, in a manner that was proportional to the amount of transfected receptor, reveal processes that are not operative with saturating concentrations of glucocorticoid. These variations also demonstrate that caution should be exercised in making mechanistic conclusions based solely on experiments conducted with saturating concentrations of glucocorticoid.

The overriding experimental advantage of transient transfections is time. Thus, the biological consequences of altered nucleotide compositions in the cDNAs encoding active proteins, and in genomic sequences, can be examined in a fraction of the time required to establish cell lines with the same sequences stably integrated into the cellular genome. In the field of steroid receptors, most of the recent advances have emerged from transient transfection experiments, including the contributions of cis-acting elements (1, 2), of different nucleotides in receptor binding to the hormone-responsive element (3), of various regions of receptors in steroid binding and biological activity (reviewed in Ref. 4), of promoter structure and cell type as determinants for the activity of antisteroid activity (5), and of overlapping signaling systems such as dopamine (6), epidermal growth factor (7), and protein kinase A inducers (8, 9). The utility of transient transfections has been further enhanced by the development of the “two-hybrid” (10, 11) and similar assays, which allows the direct detection of the interactions of two (or more) proteins (12, 13).

Most transient transfection assays with steroid receptors concentrate on the responses with saturating concentrations of activating ligand. A common problem is that a limiting factor(s) may be sequestered by the transfected DNA or the overexpressed protein encoded by the transfected DNA. In transcriptional activation assays, this behavior is called squelching or titration interchangeably (14). For the purposes of this paper, we distinguish between titration and squelching as follows. If increasing amounts of transfected receptor cDNA initially cause an increase in activity followed by a plateau, then the transfected DNA or expressed protein can be shown mathematically in a simple model of transcriptional activation to be complexing with only one necessary factor of limiting amounts. We call this behavior titration. If, however, two or more limiting factors are being removed from the system, then the behavior will be that of an initial increase in activity followed by a decline. We call this behavior squelching. While the problems of titration and squelching usually can be avoided by reducing the amount of transfected cDNA, the existence of these phenomena are informative in that they reveal the involvement of otherwise unsuspected components.

In transient transfection assays with steroid receptors, it is uncommon to examine the behavior of subsaturating concentrations of ligand despite the fact that such assays would more closely mimic the properties of gene induction by physiological concentrations of steroid. This neglect is usually due to the larger signal/noise ratios afforded by saturating ligand concentrations and the reasonable assumption that mechanisms operative with saturating and subsaturating concentrations of ligand will be the same. However, this premise may be incorrect. In fact, it has already been established that a cis-acting element of the rat tyrosine aminotransferase gene, called a glucocorticoid-modulatory element (2, 15, 16), can cause a left shift in the dose-response curve by modifying the induction properties of subsaturating concentrations of glucocorticoids without altering the fold induction by saturating concentrations of the same steroid (17). Thus, the glucocorticoid-modulatory element provides a mechanism by which the same physiological concentration of receptor-steroid complex can cause different levels of expression in various genes.

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This paper describes studies of another system that gives the same type of left shift in the dose-response curve without affecting the induction by saturating concentrations of ligand. These results were observed in experiments with a standard glucocorticoid-responsive reporter gene where the amount of transiently transfected glucocorticoid receptors was varied. Thus, the steroid induction properties of a transiently transfected gene were found to be sensitive to the concentration of steroid receptor. At the same time, the amount of agonist activity displayed by anti-glucocorticoids was found to increase.

**MATERIALS AND METHODS**

Unless otherwise indicated, all operations were performed at 0 °C. **Chemicals**—Chemicals were commercially available from the following suppliers: [14C]chloramphenicol (55–60 mCi/mmol) and promegestone (R5020), DuPont NEN; HEPES (free acid, Ultrol grade), Calbiochem; CaCl2, Fisher; dexamethasone (Dex), Sigma; PBS (Mg2+ and Ca2+ free), Quality Biological (Rockville, MD); restriction enzymes, Life Technologies, Inc., and New England Biolabs (Beverly, MA); Boehringer Mannheim or Stratagene (La Jolla, CA); acetyl-CoA, Sigma, Boehringer Mannheim, or Calbiochem; Hydrofluor, National Diagnostics (Manville, NJ); pBluescript SK+ (+), Stratagene; and pECl CMV expression vector, INVITROGEN. RU 486 was a gift from Etienne Baulieu (Paris, France).

**Plasmids**—GREtkCAT, which was originally named PRE-PBL7 and provided by Bert O’Malley, contains two inverted repeats of the 23-base pair glucocorticoid response element (GRE) II of the tyrosine aminotransferase gene in front of the thymidine kinase promoter driving the chloramphenicol acetyltransferase (CAT) gene (17, 18). pSVhGR (19) was donated by Renco A. Spanjaard. VARO1-525 (20) was a gift from Keith Yamamoto. pSVL1-766, pdhfr537-766, and pdhfr550CR732Q was donated by Renco A. Spanjaard. VARO1-525 (20) was a gift from Keith Yamamoto. pSVL1-766, pdhfr537-766, and pdhfr550CR732Q have been described (21).

pSVL was obtained by digesting pSVLGR (22) (a gift from Keith Yamamoto) with BamHI to excise the rat glucocorticoid receptor cDNA and relaxed to itself. pSVLGR4-445 was prepared by replacing the SalI/XhoI fragment from pSVLGR with the SalI/XhoI fragment from Z4-445 (23). The XhoI and XhoI restriction sites were filled in by incubation with Klenow fragment before digestion with SalI. pSVL407-795 was made by removing the 407–795 sequence as a BamHI fragment from VARO407-795 (23) and inserting it into the empty pSVLT vector obtained by BamHI digestion of pSVLGR with the same restriction fragment from dhfr550CR732Q (21). Similarly, pSVLGR/CMV and pSVLGR/CS1/CD were prepared by swapping the BamHI fragment from pSVLGR with the analogous fragments from pSTCGRN75-CS1 and pSTCN795-CS1-CD (25). The BamHI fragment from pCMV-GR-EGA (donated by Leonard Freedman) was the same fragment from pSVLGR to afford pSVLGR/EGA. pSVLNPRI was made by placing the BamHI/Asp-700 fragment of hPRI (26) into the expression vector pSVL after linearization with BamHI.

Enzymatic manipulations were performed according to the supplier’s recommendations. The constructions were transformed in DH5α competent cells (Life Technologies, Inc.), and plasmid DNAs were extracted and purified by a Qiagen (Chatsworth, CA) procedure, using their Maxi Kit. All plasmids were verified by restriction enzyme digestion, and the point mutation in pSVLGR/732Q was confirmed by sequencing.

**Cell Culture and Transient Transfections**—Monolayer cultures of HeLa cells (epithelial adenocarcinoma from human cervix; gift of Gordon Hager (NIH) or CV1 cells (African green monkey kidney from American Type Culture Collection, Rockville, MD) were plated at a density of 5 × 10^5 cells or 2.5 × 10^5 cells/60-cm dish, respectively, in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 1% heated-inactivated fetal calf serum (BioWhittaker, Walkersville, MD) and maintained in 5% CO2 atmosphere at 37 °C. Triplicate dishes were transfected by the calcium phosphate method with 2 μg of GREtkCAT receptor ± receptor plasmid with the total amount of DNA being adjusted to 3 μg with pBluescript SK+ (+) (Stratagene). Cells were incubated, harvested, and lysed, and the CAT enzyme activity was determined as described (16).

**Analysis of Data from Transient Transfections and Statistics**—The fold induction by Dex was calculated as the CAT activity (pmol/mg protein/min) with 1 μM Dex divided by the basal activity obtained with ethanol. The CAT activity was expressed as a percentage of maximal activity with 1 μM Dex. Individual values were generally within ±20% of the average, which was plotted. Unless otherwise noted, all statistical analyses were performed using the program InStat 2.03 for Macintosh (GraphPad Software, San Diego, CA).

**RESULTS**

**Transiently Transfected Receptors Alter the Kinetic Properties of Endogenous HeLa Cell Receptors**—The endogenous glucocorticoid receptors of human HeLa cells are sufficient to mediate glucocorticoid induction from the transiently transfected receptor construct GREtkCAT. However, co-transfection of rat glucocorticoid receptors caused a left shift in the dose-response curve of the potent glucocorticoid dexamethasone (Dex) (Fig. 1). Thus, the average induction by 1 nM Dex as a percentage of maximal induction increased from 19 ± 9% to 64 ± 20% (± S.D., n = 16, p < 0.0001) by adding receptor cDNA. At the same time, the amount of agonist activity exhibited by saturating concentrations of the antiglucocorticoid dexamethasone 21-mesylate (Dex-Mes) jumped from 4 ± 2% to 49 ± 17% (± S.D., n = 16, p = 0.0001) of the maximal activity induced by saturating concentrations of Dex. Thus, the presence of transfected receptors converted Dex-Mes from an almost pure antiglucocorticoid to a partial agonist. No change in the activities of 1 nM Dex and 1 μM Dex-Mes was seen when HeLa cells were transfected with the empty expression vector pSVL (data not shown). Therefore the behavior in Fig. 1 is mediated by overexpressed receptors.

The effects of transfected receptors were usually obtained with little or no increase in the absolute CAT activity of the basal or fully induced levels (Fig. 1). Others have reported increased CAT activities after transient transfection of glucocorticoid receptors into HeLa cells, but a different reporter
We found that the percentage of agonist activity of 1 nM Dex and 1 μM Dex-Mes increased approximately linearly up to 0.4 μg of receptor cDNA (Fig. 2A). This receptor-dependent increase in the percentage of agonist activity without a concomitant increase in the fully induced CAT activity at 1 μM Dex is not thought to reflect a titration of a limiting factor(s) by the high receptor/reporter ratios for three reasons. First, in those experiments where the transfected receptor did cause higher levels of CAT activity with 1 μM Dex, the increase in the percentage of agonist activity with 1 nM Dex or 1 μM Dex-Mes was just as pronounced (percentages of agonist activity for 1 nM Dex and 1 μM Dex-Mes were 12 and 2%, respectively, of the maximal activity of 2397 dpm without transfected receptors versus 55 and 36% of the maximal activity of 15,262 dpm with transfected receptors). Second, the absolute CAT activity with saturating Dex concentrations always increased with additional reporter (Fig. 2B). Third, in a different system of CV-1 cells, where the absolute CAT activity at 1 μM Dex was proportional to added receptor, the percentage of agonist activity with 1 nM Dex or 1 μM Dex-Mes still increased (Fig. 2C).

Altered Kinetic Properties of Glucocorticoid Induction Are General for Receptor, Cell Type, and Steroid—All results in HeLa cells were obtained when adding transfected rat glucocorticoid receptors to human glucocorticoid receptors. However, transiently transfected human and rat receptors were equally active in HeLa cells (Table I). Therefore, the observed results do not involve some molecular conflict between receptors of different species.

The experiments of Fig. 2C in receptorless CV-1 cells further allowed us to ask whether a conflict between endogenous and exogenous receptors contributes to the increased activities of 1 nM Dex and 1 μM Dex-Mes. As shown, larger amounts of rat receptor cDNA afforded a dramatic increase in the percentage of agonist activity displayed by 1 nM Dex or 1 μM Dex-Mes. Thus, the increased activity of these suboptimal inducers occurred even when almost all of the receptors are transiently expressed and does not require an interaction or competition between endogenous and exogenous receptors. The results of Fig. 2C also establish that the effect of transfected receptors is not unique to HeLa cells but can be observed in cells from another tissue and species (i.e., monkey kidney cells).

The most dramatic increases in agonist activity in HeLa cells were usually seen with 1 μM Dex-Mes (Fig. 2). To determine
TABLE I

| Receptors                  | No. of exp. | Activity with 1 μM Dex | Activity with 1 μM Dex-Mes | % of maximal induction by 1 μM Dex |
|----------------------------|-------------|------------------------|---------------------------|-----------------------------------|
| Endogenous                | 5           | 11 ± 3                 | 4 ± 1                     | 11%                                |
| Transfected hGR           | 3           | 19 ± 4                 | 13 ± 2                    | 19%                                |
| Transfected hGR plus CD   | 3           | 21 ± 4                 | 15 ± 2                    | 21%                                |

whether this might result from the unique properties of Dex-Mea as an affinity-labeling steroid (29, 30), the properties of the antagonist progesterone and the "pure" antiglucocorticoid RU 486 were examined. Marked increases in agonist activity were observed with each antagonist (Fig. 3). Therefore, both covalent and noncovalent antiestrogens acquire substantial amounts of agonist activity simply when assayed in the presence of transiently transfected glucocorticoid receptors.

Effect of Transiently Transfected Receptors Requires Entire Protein in a Form That Is Biologically Active—Numerous glucocorticoid receptor deletion (Fig. 4A) and substitution mutants were selected to identify which region(s) of the transiently transfected receptor was required to evoke the above responses. Surprisingly, no one segment of the rat receptor retained significant activity. Even with the lower amount of transfected receptors (0.2 μg), receptors lacking either the amino-terminal domain (i.e. 407C = amino acids 407–795) or the DNA-binding domain and the steroid-binding domain (i.e. N445 = amino acids 1–445) were almost inactive (Fig. 4B). The weak effect of transfected 407C receptors in the presence of 1 μM Dex-Mes (p = 0.049 relative to endogenous glucocorticoid receptor) could be due to the low (−10%) activity of this (data not shown) and homologous (31, 32) receptor fragments in CV-1 and COS-7 cells. Others have found that full biological activity of the glucocorticoid receptor could be reconstituted when the two halves, fused to jun and fos molecules, were brought together by the leucine zipper (19). However, the simple addition of the two halves, in the form of N445 and 407C, was unable to reproduce the effect of the full-length receptors. Even N766, which contains all but the carboxyl-terminal 29 amino acids of the receptor, was inactive (Fig. 4B). The properties of the constitutively active N525 fragment were difficult to assess due to the very high basal levels and minimal steroid induction of CAT activity (data not shown).

N766 receptors, like N767 (25), did not bind Dex but were affinity labeled by [3H]Dex-Mes (data not shown). This, plus the inactivity of 407C, suggested that steroid binding to transfected receptors is not sufficient and that the steroid-bound receptors must possess good biological activity to elicit the observed effects. This hypothesis was examined in greater detail with several point mutations that have altered biological activity. Replacement of the three amino acids Gly, Ser, and Val at the distal base of the first zinc finger of the glucocorticoid receptor with Glu, Gly, and Ala, respectively, has been found to redirect receptor binding from a GRE to an estrogen-responsive element, there is disagreement in the literature as to whether the specificity is absolute (Ref. 33 versus Ref. 34). We observed no biological activity with receptors containing the GSV to EGA replacement (GREga) after transfection into CV-1 cells (data not shown), consistent with no DNA binding. Nevertheless, transfection of the same GREga receptors into HeLa cells significantly increased the activity of 1 nM Dex and 1 μM Dex-Mes (Fig. 4C). Thus, receptors unable to bind to the GRE do retain activity upon transfection, albeit less than the wild-type receptors. Furthermore, while DNA binding to the GRE is not necessary, the activity of transfected receptors do require not only virtually all of the receptor protein but also a conformation of the receptor that is capable of independent transcrational activation, which has been seen here only after the binding of appropriate ligands.

Effect of Transiently Transfected Receptors Is Specific for Glucocorticoid Receptors—We next inquired whether the above effect of transfected glucocorticoid receptors could be evoked by another receptor. We chose progesterone receptors not only because of their high overall homology with glucocorticoid receptors (35) but also because the smaller A-form of human progesterone receptors (PR-A) has been found to repress the induction by several receptors including the glucocorticoid receptor under conditions where PR-A itself is either inactive (36) or active (37). Addition of up to 10 nM R5020 to HeLa cells transfected with the GREkCAT reporter afforded no induction, consistent with the absence of endogenous progesterone receptors, while a 7-fold induction was seen with co-transfected human PR-A receptors (data not shown). However, the activi-
ties of 1 nM Dex and 1 μM Dex-Mes were unaffected by co-transfected PR-A receptors either in the presence or absence of 10 nM R5020 (Fig. 5). The partial diminution of the effect of transfected glucocorticoid receptors in the presence of R5020 (Fig. 5) is presumably due to the antiglucocorticoid activity of R5020 (38). Thus, the ability of transfected receptors to increase the activities of 1 nM Dex and 1 μM Dex-Mes is somewhat specific and is not shared by progesterone receptors. Furthermore, the presence of steroid-free PR-A had no effect on the fold induction by glucocorticoid receptors in the presence of 1 μM Dex (fold induction with PR-A/without PR-A = 1.22 ± 0.34 [S.D., n = 6]).

DISCUSSION

Considerable mechanistic information has emanated from experiments with transiently transfected steroid receptors. However, the present study shows that two of the induction properties of a common reporter gene (GREtkCAT) with transiently transfected receptors are, surprisingly, not invariant. Thus, the agonist activity of both saturating concentrations of antagonists and of subsaturating concentration of agonist (i.e. a left shift in the dose-response curve) increased in a manner that was directly proportional to the total concentration of receptors. These phenomena were independent of the cells used, the species of glucocorticoid receptors, the presence of endogenous receptors, the concentration of transfected reporter gene, and the chemical nature of the antiglucocorticoids. No one region of the transfected receptors was sufficient to increase these induction parameters. Rather an intact receptor in a transcriptionally active, steroid-bound form was required for activity, although DNA binding to the GRE was not necessary.

Almost all reported studies with transfected glucocorticoid receptors have focused on the fold induction with saturating concentrations of agonists and of subsaturating concentrations of agonist (i.e. a left shift in the dose-response curve) increased in a manner that was directly proportional to the total concentration of receptors. These phenomena were independent of the cells used, the species of glucocorticoid receptors, the presence of endogenous receptors, the concentration of transfected reporter gene, and the chemical nature of the antiglucocorticoids. No one region of the transfected receptors was sufficient to increase these induction parameters. Rather an intact receptor in a transcriptionally active, steroid-bound form was required for activity, although DNA binding to the GRE was not necessary.

Almost all reported studies with transfected glucocorticoid receptors have focused on the fold induction with saturating concentrations of agonists in cells lacking endogenous receptors, thus making comparisons with the present data difficult. One similarity, though, is the relatively high levels of agonist activity of the antiglucocorticoid RU 486 that has been noted with transfected glucocorticoid receptors (Fig. 3B; Refs. 39 and 40).

Overexpression of transcription factors like the glucocorticoid receptor can result in the titration or squelching of other transcription factors. With the glucocorticoid receptor, squelch-
Transfected glucocorticoid receptors to cause increased amounts of progesterone receptors (46). However, the ability of transiently transfected receptors to cause increased dimer formation in solution and precocious transactivation. Thus, higher levels of receptors following the transfection of receptors could cause increased dimerization in solution and precocious transactivation. However, no dimerization of the glucocorticoid receptor DNA-binding domain was observed even under the very high protein concentrations used for NMR studies (44). Also, dimerization has been reported not to require the steroid-binding domain (45), while the transfected glucocorticoid receptors must be intact and functionally active if they are to alter the activity of 1 nM Dex or 1 μM Dex-Mes (Fig. 4). Finally, the effects of transfected receptors were seen even with mutant GREga receptor that cannot bind to (33) or activate a GRE (Fig. 4 and data not shown). Thus, increased receptor dimerization appears an unlikely cause.

Because all of the data are expressed as a percentage of the maximal induction by 1 μM Dex, any mechanism involving changes in the chemical nature of the translated receptor, or of some other regulatory protein, should affect all receptors equally and can be discounted. An intriguing alternative possibility is that there is some difference, or conflict, between endogenous and transiently transfected receptors, similar to the unequal activation of stably replicated mouse mammary tumor virus templates by transiently versus stably transfected progesterone receptors (46). However, the ability of transiently transfected glucocorticoid receptors to cause increased amounts of agonist activity for 1 nM Dex and 1 μM Dex-Mes either without (Fig. 2C) or with (Figs. 1 and 2A) endogenous receptors that are or are not homologous (Table I) seems to eliminate this possibility.

The most likely explanation is that the transfected receptors are interacting with one or more transcription factors in a manner that does not require receptor binding to DNA. One class of factors would be components of the initiation complex that have been found to interact with steroid receptors, such as the transcription factor IID-associated factor TAFII-30 (TATA-binding protein-associated factor II-30) (47), transcription factor IIB (48), or transcription intermediary factor 1 (TIF1), which is not associated with TATA-binding protein, transcription factor IID, or transcription factor IIB but is reported to cause a left shift in the dose-response curve of RXR (49). TIF1 was also found to interact with the hormone-binding domain (HBD) of estrogen (ER) and progesterone receptors but did not cause increased agonist activity for a VP16/ER-HBD fusion protein bound with the antiestrogen 4-hydroxytamoxifen. Alternatively, the receptors could be complexing a repressor. Transcription activation functions in general have been proposed to act as inhibitors (50), and reduced concentrations of SPT6, which is active in yeast and mammalian cells, caused a left shift in the estrogen dose-response curve (51). Genetic deletion of the yeast repressor SSN6 also caused increased percentages of agonist activity for antiestrogens, but not anti-progestins, and a >10-fold left shift in the dose-response curve for agonists bound to estrogen or progesterone receptors in yeast (52). Unfortunately, no mammalian homologues of SSN6 have yet been described. Finally, the putative factor could determine the “coupling efficiency” of other transcription complex components as this behavior can cause a left shift in the dose-response curve (53, 54). Each model implicates a potentially novel factor(s) in regulating the amount of antisteroid activity expressed by an antiglucocorticoid and in setting the position of the dose-response curve. We further conclude that if titration (or squelching) of an inhibitory factor(s) of the transcriptional machinery does occur, this factor(s) does not regulate the basal or fully induced levels of transcription, which are unchanged in Figs. 1 and 2A.

A commonly used method of identifying auxiliary factors interacting with transcription factors is the yeast two-hybrid assay (10). However, we will have to use a mammalian selection procedure (55) to screen for our putative factor(s) due to the aberrant behavior of Dex and antiglucocorticoids in yeast (56, 57).

The defined conditions of our assay with the simple GREtk-CAT construct have revealed another mechanistic component of steroid receptor-activated gene transcription that might act in conjunction with the previously described cell-specific and promoter-specific factors (5) and/or with other interacting signaling systems, such as dopamine (6), epidermal growth factor (58), and protein kinase A (59, 60). One major difference with the above influences on steroid hormone action is that only transfected receptors have yet been shown to cause both increased agonist activity among antisteroids and a left shift in the dose-response curve of agonists. It remains to be seen whether the mechanism of action of the glucocorticoid-modulatory element, which also modulates the properties of antagonists and agonist dose-response curves (2, 15, 16), shares a common component or is related.

Androgen, mineralocorticoid, progesterone, and glucocorticoid receptors all bind to, and activate transcription from, the same hormone response element. However, this mechanistic commonality does not extend to the present effect of transfected receptors since progesterone receptors, either with or without R5020, were unable to alter the induction properties of the endogenous glucocorticoid receptors of HeLa cells (Fig. 5). Thus, there are other components that are specific at least for glucocorticoid versus progesterone action that may dictate how steroid-specific gene regulation can occur from seemingly identical hormone response elements.

The lack of activity of PR-A in our system is of interest in view of the reports that PR-A is a dominant negative inhibitor of the steroid-mediated induction by many other receptors, including glucocorticoid receptors (36, 37). While the transfected PR-A receptors were active in CV-1 cells in the presence of 10 nM R5020, they did not reduce the fold induction by 1 μM Dex-bound glucocorticoid receptors in HeLa cells. Thus, as has been observed by others (61), the ability of PR-A to be a dominant repressor may be promoter- and/or cell-specific.

In conclusion, different components of the glucocorticoid receptor-mediated induction pathway can be rate-limiting under different experimental protocols. This can significantly alter the results and mechanistic interpretations. For example, we found that some kinetic properties of steroid-regulated induction are proportional to the concentration of transcriptionally active receptors. These results implicate new components in glucocorticoid-regulated gene expression, and determinants of agonist activity for antiglucocorticoids, that should be profitable areas of future investigations.

Acknowledgments—We thank Gary Felsenfeld (NIH) for helpful comments, Etienne Baulieu (Paris, France) for RU 486, Pierre Chambon (Strasbourg, France) for hPR1, Len Freedman (Memorial Sloan-Kettering Cancer Center) for pCMV-GR-EGA, Gordon Hager (NIH) for HeLa cells, Bert O'Malley (Baylor College of Medicine) for PRE-PBL7, Sandro Rusconi (University of Freiburg) for pSTCGRN795-CS1 and...
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pSTCN795-C51-CD, Renco Spanjaard (Brigham and Women's Hospital) for pSVhGR, and Keith Yamamoto (UCSF) for pSVLGR and Varol-525.

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