Correlation Among Agonist Dose, Rate of Import, and Transcriptional Activity of Liganded Progesterone Receptor B Isoform in Living Cells

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Purpose. To determine the correlation among progesterone dose, rate of import of progesterone-occupied progesterone receptor (PR) complexes into the nucleus of cells, and transcriptional activity of progesterone–PR complexes.

Methods. Live cell imaging and time-lapse microscopy of green fluorescent protein-tagged PR were performed to measure the rate of import into the nucleus of progesterone–PR complexes. To measure transcriptional activity, a progesterone-PR–sensitive luciferase reporter gene assay was used.

Results. For low doses of progesterone, there was a correlation among dose, import into the nucleus, and transcriptional activity. At higher doses of progesterone (beyond 12.5 nM), transcriptional activity increased, but there was no further increase in the rate of import, indicating a saturation of the import machinery. In both cases, a simple one-compartment model was sufficient to describe the import data.

Conclusions. At low doses, progesterone dose correlates well with rate of import and transcriptional activity. At high doses, more progesterone can get into the nucleus and can activate unoccupied receptors already in the nucleus, leading to higher transactivation than would be expected from rates of import of progesterone–PR complexes into the nucleus.

KEY WORDS: progesterone; luciferase assay; fluorescence intensity; rate of import; nucleus; progesterone receptor.

INTRODUCTION

Cellular kinetics of the transport of drug–receptor complexes to the nucleus is a new area of study that converges the fields of pharmaceutics, pharmacology, and cellular and molecular biology by utilizing novel technologies to measure the rates of import into living cells of drug–receptor complexes. Our work focuses on the human progesterone receptor (PR), a member of the nuclear receptor superfamily that includes steroid receptors.

Steroid receptors have been extensively studied biochemically and histologically but have not until recently been studied on the subcellular level in living cells (1,2). Steroid hormone receptors are transcription factors that have the capability of shuttling between the nucleus and cytoplasm. Characterizing nucleocytoplasmic trafficking is essential to understanding receptor/gene-mediated kinetics and dynamics at the cellular level. The localization of PR in the absence of ligand reflects a dynamic situation: the receptors are shuttled continuously between cytoplasm and nucleus (3). Progesterone receptors can act genomically on transfer into the nucleus after ligand binding. Once in the nucleus, they activate their target genes.

Although classical pharmacokinetic and pharmacodynamic studies have been done for steroid hormone receptor agonists and antagonists, the study of the rate of steroid receptor transport into nucleus after ligand binding, trafficking between cytoplasm and nucleus, and the consequent gene transcription is intriguing from a cellular pharmacokinetic and pharmacodynamic point of view. These types of cellular studies reveal important new information on what drug–receptor complexes are doing at the cellular level. Such information may include correlating the dose of drug with rate of import into the nucleus and, subsequently, the transcriptional activity.

Progesterone receptor B isoform (PRB) was chosen as a model to study because of its localization in living cells (nuclear and cytoplasmic) and because of its well-documented transcriptional activity using reporter gene assays (1). PRB was found to localize predominantly in the nucleus based on immunohistochemical techniques on various reproductive tissues and cells (4,5). Recently, with the development of green fluorescent protein (GFP), PRB was found to localize in both the nucleus and cytoplasm (although on average still mainly nuclear) (1). Using fluorescence microscopy, the enhanced version of green fluorescent protein (EGFP) ligated to the N-terminal of PRB has been visualized in living mammalian adenocarcinoma cells in real time. For this paper, the import kinetics of progesterone-occupied progesterone receptor were explored and correlated with the "dynamic process"—gene transcription.

MATERIALS AND METHODS

Transient Transfections and Cell Culture

Mouse adenocarcinoma 1471.1 cells (a kind gift from G. Hager, NIH) were maintained in Dulbecco's modified Eagle's medium (DMEM, GIBCO-BRL, Grand Island, NY) with 10% fetal bovine serum (FBS, HyClone Laboratories Inc., Logan, UT) in 175-cm^2 flasks at 37°C with 5% CO_2. 1471.1 cells do not express endogenous progesterone receptor.

Two micrograms of EGFP-PRB plasmids (6) were transiently cotransfected with either 8 μg carrier DNA (pGL3basic) for microscopy studies or 10 μg of pMMTV-luc (firefly luciferase, G. Hager, NIH) and 20 ng pRL-SV40 renilla luciferse (Promega Corp., Madison, WI) for luciferase assays into 5×10^6 1471.1 cells via electroporation. Electroporations were performed at 135 V, 10 ms, and three pulses as

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ABBREVIATIONS: DMEM, Dulbecco's modified eagle's medium; EGFP-PRB, enhanced green fluorescent protein linked to the human progesterone receptor B isoform; FBS, fetal bovine serum; FLIP, fluorescence loss in photobleaching; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; GTP, guanosine triphosphate; NLS, nuclear localization signal; NPC, nuclear pore complex; PBS, phosphate-buffered saline; PR, progesterone receptor; PRB, progesterone receptor B isoform; ROI, region of interest.

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previously described (6). After transfection, approximately one sixth to one quarter of the total cells were plated onto individual living cell chambers for microscopy (Lab-Tek II chambered coverglass with cover #1.5, Nalge Nunc International, Naperville, IL); or one 12th of the total cells were plated into individual wells of a six-well plate for luciferase assays in DMEM with 10% charcoal-treated FBS at 37°C.

Luciferase Assay

The Dual Luciferase Reporter Assay System® (Promega Corp., Madison, WI) was used to determine firefly luciferase activity (to measure EGFP-PRB transactivation of pMMTV-luc) and renilla luciferase activity (as a control) according to the manufacturer's instructions. Firefly luciferase activity was normalized to renilla luciferase activity in all graphs shown.

Hormone, Hormone Induction, and Washing (Withdrawal of Hormone)

Progesterone was purchased from Sigma Chemical Co. (St. Louis, MO). Progesterone stock solutions in absolute ethanol were added to cells (in living cell chambers) 24 h after transfections to make the desired final concentrations (0, 0.1, 1, 12.5, 100, or 1000 nM).

For hormone inductions for the luciferase assay, cells were induced with a given hormone dose for 5, 10, 20, 30 min and 6 h. For any given time point, hormone was added for that particular amount of time, then removed by washing five times with phosphate-buffered saline (PBS) before the addition of fresh medium. To allow for maximal luciferase expression, luciferase assays were performed after 6 h of the start of the inductions. For hormone inductions for the microscopy studies, simple time-lapse experiments were performed (i.e., no washing out of hormone).

For the hormone withdrawal experiments (to measure detectable export), after a 2-h incubation of the cells with hormone, the medium was washed away with PBS five times, then fresh medium was added to the chamber. Images were taken at 2, 9, and 24 h. For the nonwithdrawal experiment, the cells were incubated with progesterone (without removing the hormone), and the images were taken at 2, 9, and 24 h.

Fluorescent Live Cell Microscopy

To visualize changes in EGFP-PRB movement on addition of ligand, fluorescence microscopy was performed using an Olympus 1 × 70 inverted system microscope (Scientific Instrument Company, Aurora, CO) on living cells following hormone treatment. Details are as follows: 24 h after transfection, the medium was changed in living cell chambers; chambers were then secured to the microscope stage. An air stream incubator (Nevtek, Burnsville, VA) and temperature probe were used to maintain a temperature of 37°C for the cell chambers. Cell chambers were allowed to equilibrate to 37°C before addition of hormone. To visualize EGFP-PRB, a high-quantity narrow-band GFP filter was used (excitation filter set HQ480/20; emission filter set HQ510/20; with beamsplitter Q495lp) from Chroma Technology Corp. (Brattleboro, VT). To minimize photobleaching of the EGFP chromophore, cells were imaged using neutral-density filters that transmit 25% of the total light and short (500-ms) exposure times. Fluorescent images were taken before hormone induction (t = 0) and at 5, 10, 20, 30, 40, 50, 60, 75, and 90 min for all progesterone concentrations. Additional images at 3 min and 15 min were taken for high hormone concentrations to catch any additional detail that occurred at high doses as a result of rapid transport.

Because of the heterogeneity of PRB localization (1) in cells, we excluded cells in which the PRB population localized exclusively in the nucleus because, in these cells, no PRB transport from cytoplasm to the nucleus could be observed using this system. Cells that showed EGFP-PRB localized predominantly in the cytoplasm or partially nuclear and cytoplasmic were chosen for this study. Cells with PRB expression that is predominantly cytoplasmic or partially nuclear and cytoplasmic do represent the majority of the cells according to Lim *et al.* (1) and our unpublished observations. Cells that are predominantly nuclear make up only a small fraction (less than 10%) of the majority (1).

Data Analysis

All images were analyzed by analySIS® software (Soft Imaging System GmbH, Lakewood, CO). AnalySIS® software allows one to draw around a region of interest and then calculates the pixel (fluorescence) intensity in that region of interest. The fluorescence intensity for nucleus and cytoplasm were measured for each cell at every time point. For a given cell, to calculate the percentage of fluorescence intensity in the cytoplasm at time point t, the following equation was used:

$$\%\text{Fl Int Cyto} = \frac{\text{Cyto Fl Int} - \text{Bkgrd Fl Int}}{\text{Total Fl Int}} * 100$$

where % Fl Int Cyto = percentage of fluorescence intensity in the cytoplasm at time t; Cyto Fl Int = cytoplasmic fluorescence intensity at time t; Bkgrd Fl Int = background fluorescence intensity at time t; and Total Fl Int = total fluorescence intensity at time t.

For each dose, the average percentage cytoplasmic intensity vs. time was plotted using SigmaPlot (SPSS, Chicago, IL). The kinetic model parameters were solved using Scientist (Micromath, Salt Lake City, UT), and the relationship between nuclear intensity and increase in transfection was fitted to a three-parameter sigmoidal relationship using nonlinear regression in SigmaPlot. The solved parameters included I_{max}, the maximum induction increase in transcription, NI_{50}, the percentage of nuclear intensity necessary for 50% of the maximum induction to occur, and sigmoid slope parameter. For analyzing differences between the model parameters, ANOVA was used with Tukey correction for multiple comparisons.

RESULTS

Ligand–Receptor Complexes Transport from Cytoplasm to Nucleus

To visualize the progesterone receptor B isoform in living cells, EGFP was fused to the N-terminal of the intact human progesterone receptor B isoform. We have shown previously that EGFP does not affect PRB localization and function and that PRB distributes in both the cytoplasm and nucleus in different cell lines (1). Addition of progesterone

(hormone agonist) to the culture medium resulted in a time-dependent transport of the EGFP-PRB into the nucleus. An example of this is shown in Fig. 1 for the 1000-nM dose of progesterone. Hormone–receptor complex transport into the nucleus was rapid and clearly evident within 5 min for high concentrations of hormone (see Fig. 1; in the 5-min photo, an increase in nuclear accumulation is apparent compared to 0 min) and occurred within 15 min for low concentrations of hormone (data not shown). The hormone–receptor complexes became predominantly nuclear within 30 min, and a plateau was achieved after 40 min except for the 0.1-nM dose, which did not reach a plateau during the time course of the experiment (see Fig. 2, 0.1 nM dose).

Correlation of Dose to Rate of Import

The range of doses of progesterone selected was from 0.1 nM to 1000 nM, which spans well beyond the normal physiologic and cancerous progesterone concentrations (between 0.5 and 100 nM). Also, the level of EGFP-PRB expressed in these cells was similar to physiologic levels found in T-47D cells (which express PRB) (1). The relationship between hormone concentration and rate of import was studied first. We determined the rate of import of hormone–receptor complexes with increasing dose. Figure 2 shows that the rate of import from the cytoplasm to the nucleus increased with increasing progesterone concentration. An average of 36 cells for each dose were selected in the graph shown in Fig. 2 ($n = 31$ at least, in all cases). It was found that import is saturated at 12.5 nM based on the fact that the intensity curves are identical at doses up to two orders of magnitude greater than this amount (Fig. 2 and Table I), and also that there is no change in the intensity rate constant after the 12.5-nM dose (Fig. 3). Figure 2 also shows that up to the point of saturation, the higher the dose, the earlier the plateau occurs. A simple

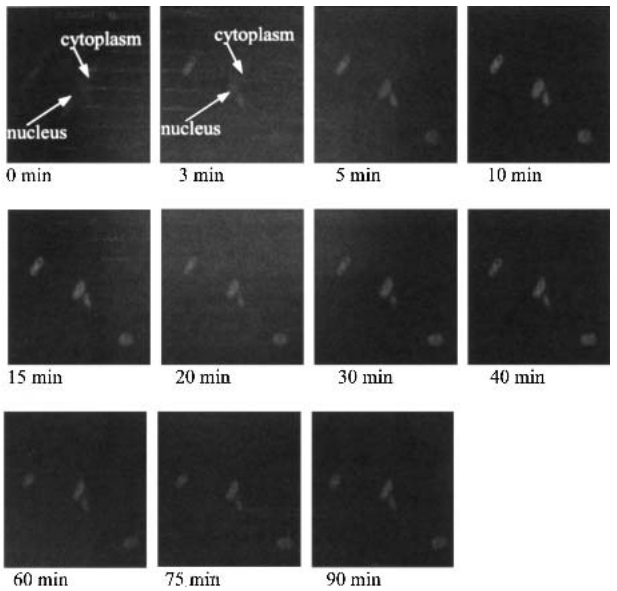

Fig. 1. Example of progesterone-dependent translocation of EGFP-PRB in living cells. Time-lapse microscopy was performed on cells expressing EGFP-PRB in the presence of 1000 nM progesterone (in this case). Import into the nucleus is clearly evident, even after only 3 to 5 min, and reaches a plateau after about 30 min. Arrows indicating cytoplasm and nucleus shown on first two time points only.

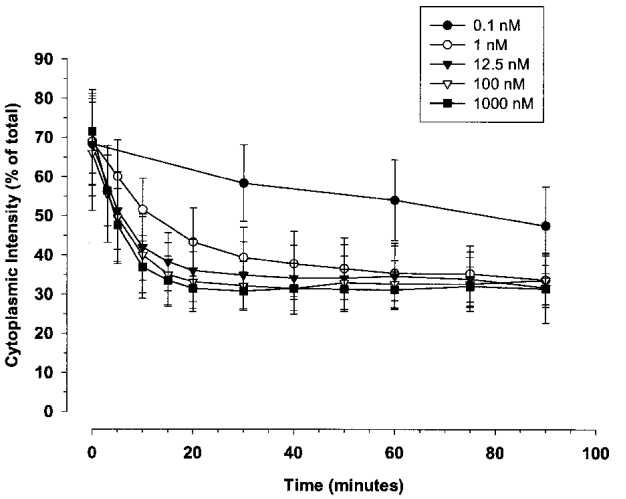

Fig. 2. Loss of cytoplasmic intensity over time. EGFP-PRB translocation into the nucleus from zero to 90 min, with different doses of progesterone (0.1 to 1000 nM doses). For each dose, $n = 31$ at least.

one-compartment pharmacokinetic model equation was found to be sufficient to describe the rate of import (see Appendix). The Tukey-Kramer test for unequal sample sizes was used to determine statistical significance of the rate constants determined for each dose (Table I). The rate of import increased in a statistically significant manner ($p < 0.001$) with increasing doses (from 0.1 nM, 1 nM, up to 12.5 nM). At higher doses, there was no statistically significant increase in the rate of import. This implies that the import machinery (import receptors and nuclear pore complex) gets saturated near 12.5 nM.

Export of PRB out of the Nucleus

In order to prove that export rate of PRB was negligible in the experimental period compared to import rate of PRB in the presence of progesterone, EGFP-PRB fluorescence was monitored after hormone treatment followed by withdrawal of hormone (by extensive washing) or nonwithdrawal of hormone (no washing). Results are presented in Fig. 4. Figure 4a shows that the export of progesterone receptor from nucleus to the cytoplasm occurs noticeably only after 9 h of hormone withdrawal. Figure 4b shows that when hormone is not withdrawn, no significant net export occurs, even after 24 h, which is well beyond the time course of the experiments shown in this paper. When hormone is not withdrawn, export occurs (5); however, the steady-state value of

Table I. Statistical Analysis of Fig. 1 Data: Calculated Change of Intensity Rate Constants (min^{-1}), Standard Deviations, and p Values

Concentration	Change of intensity rate constant (min^{-1})	Standard deviation
0.1 nM	0.01	±0.008*
1 nM	0.076	±0.036†
12.5 nM	0.164	±0.100*†
100 nM	0.171	±0.065*†
1000 nM	0.202	±0.069*†

* $p < 0.001$ compared to 1 nM value.
† $p < 0.001$ compared to 0.1 nM value.

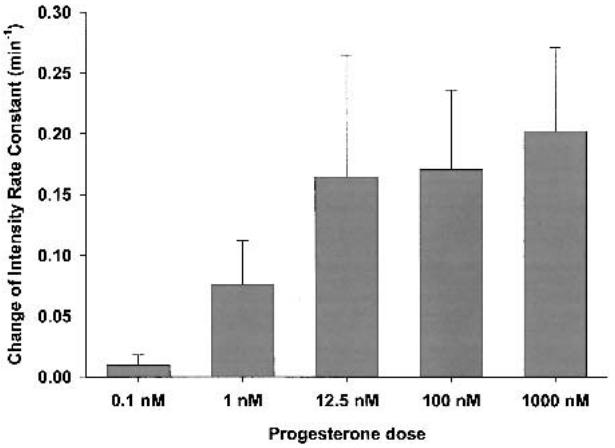

Fig. 3. Change of intensity rate constant (slope) vs. progesterone dose. After 12.5 nM, there is no further increase in the intensity rate constant, indicating no increase in the rate of import into the nucleus.

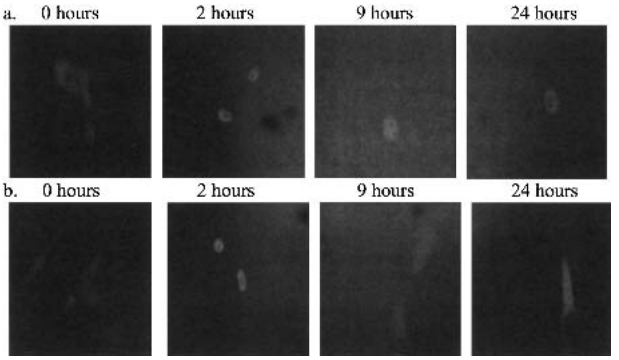

Fig. 4. Export of EGFP-PRB from the nucleus to the cytoplasm after the addition of 1 nM progesterone. Images are not time-lapse (the same cell is not tracked over time). (a) Hormone is not removed from the system; export does not occur to any significant extent, even after 24 h. (b) Hormone is removed from the system by extensive washing, and export of EGFP-PRB is observed over a 24-h period. Net export still is not apparent until the 9-h time point.

fluorescence intensity is unchanged (i.e., rate of import equals the rate of export at steady state). The constitutively active and hormone-inducible nuclear localization signals, in the presence of hormone, overcome nuclear export signals in PR. When hormone is washed out, as in Fig. 4a, significant net export does occur. Therefore, any possible net export that may occur during the time course of our nonwashout experiments is negligible. For simplicity, therefore, we ignore export in our one-compartment model.

Transcriptional Activity of PRB

The transcriptional activity of PRB increases with increasing agonist (progesterone) concentration and is saturated at around 1000 nM of progesterone (7). Fig. 5 shows transcriptional activity increasing not only with dose but with time (in the first 30 min at least). For the 0.1-nM and 1-nM doses, the transcriptional activity increases most rapidly in the first 30 min (see slopes) and tapers off after 30 min. For 12.5, 100, and 1000 nM the transcriptional activity reaches a maximum at 30 min and maintains a plateau beyond the 30-min time point. The transcriptional activity increases in the first 30 min for all doses shown correlate with the increase in import of drug–receptor complexes, at least for the 0.1-, 1-, and 12.5-nM doses. Transcriptional activity in this system depends on many factors, including dose of drug, receptor occupancy, protein synthesis (indicated by luciferase), rate of import of drug–receptor complexes into the nucleus, binding to DNA (reporter gene in this case) and activation of the reporter, and activation of receptors already in the nucleus.

Correlation between the Fluorescence Intensity in the Nucleus and the Transcriptional Activity

Because the transport of PR complexes into nucleus plateaus after 30 min, and the transcriptional activity of drug–receptor complexes is greatest in the first 30 min, the correlation between the fluorescence intensity in the nucleus and the luciferase activity during this time period was studied. For all doses, there is a nonlinear relationship between intensity (a measure of transport) and activity, as shown in Fig. 6. The correlation coefficients (r^2 values) are shown on the graph in Fig. 6. With increasing dose size, both the value of I_{max} and NI_{50} increase. I_{max} is the maximum induction increase in transcription; NI_{50} is the percentage of nuclear intensity necessary for 50% of the maximum induction to occur. The I_{max} values were 5.6, 11.9, 13, and 34.4 for the 1-, 12.5-, 100-, and 1000-nM doses, respectively (Fig. 7a). For the lowest dose of 0.1 nM, there were not enough data to adequately fit the model. This increase in I_{max} was statistically significant between the 1000-nM dose and the three lower dose groups ($p < 0.05$). The NI_{50} values were 42.8, 49.2, 44.3, and 62.6 for the 1-, 12.5-, 100-, and 1000-nM doses, respectively (Fig. 7b). The increase in NI_{50} was significant only at the highest dose. The sigmoid slope value was between 5 and 6 for all four doses and was not significantly different. At higher doses, more of the lipophilic progesterone drug is present in the nucleus and thus can activate unoccupied receptors already in the nucleus (recall that much of PRB is already nuclear to begin with even in the absence of drug). This results in a much higher response, or transactivation, as shown by the increase in I_{max} with dose. The increase in NI_{50} at the highest dose also supports the hypothesis that more drug is present in the nucleus at higher doses. With more drug in the nucleus, the NI_{50} value will

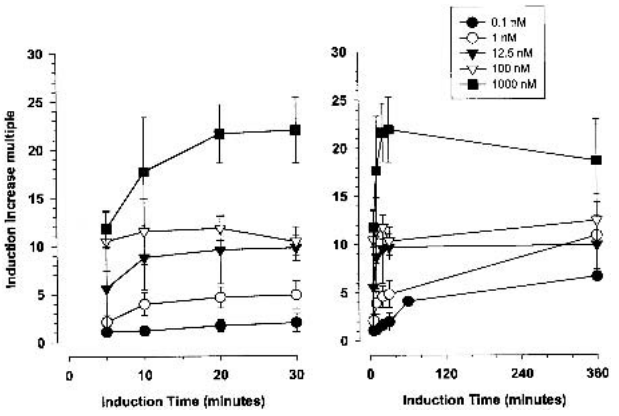

Fig. 5. Transcriptional activity of EGFP-PRB measured by luciferase reporter gene assay. For each time point, hormone was removed from the system by washing, and 6 h was allowed to elapse before luciferase readings were taken. As dose increases, transcriptional activity (fold induction) increases.

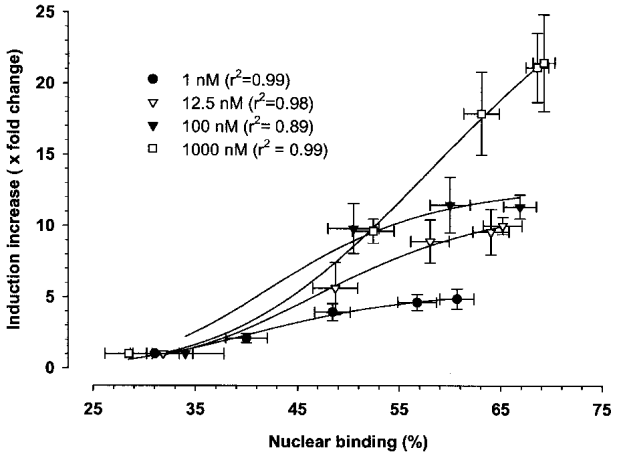

Fig. 6. Correlation between transcriptional activity (fold induction) and nuclear import (measured by percentage of nuclear intensity). The relationship between nuclear intensity and increase in transfection was fitted to a three-parameter sigmoidal relationship using nonlinear regression. Nonlinear sigmoid fit and bidirectional standard error shown.

necessarily increase because more receptor binding is occurring in the nucleus. At lower doses of progesterone, not as much progesterone is present in the nucleus, resulting in lower transcriptional activity (fold induction).

DISCUSSION

The ability to perform time-lapse microscopy of steroid receptors in living cells allows the monitoring of import of drug–receptor complexes into the nucleus. Other slightly more sophisticated techniques such as FRAP and FLIP (fluorescence recovery after photobleaching and fluorescence loss in photobleaching) of other protein molecules in the cell are also available. These techniques are able to measure fast movement of molecules in individual compartments of the cell. For our purposes, time-lapse imaging is effective because the movement of drug–receptor complexes from the cytoplasm to the nucleus occurs on the time scale of minutes rather than seconds or milliseconds (for FLIP and FRAP).

Steroid receptors have been well studied biochemically and histologically. Because of advances with green fluorescent protein technology, kinetic and dynamic studies of receptors (and other proteins) are now possible. The study of import of receptors such as PR, in the presence of drug (agonist) is one of the main focuses of our laboratory. The regulation of import and export of PR is of particular interest in terms of reproductive cancers that are PR positive.

The mechanism for PR complex import into the nucleus is believed to be an active process. PR contains two nuclear localization signals, one that is constitutively active and one that is induced by hormone. Although the constitutively active NLS is always "on," the location of PR in the unliganded (no drug) state is not completely nuclear (not homogeneous when individual cells are compared) because of export mechanisms, which have not been fully elucidated. It is, however, known that PR shuttles between the nucleus and the cytoplasm in the unliganded state and that unliganded PR can be found (at any given time) in both the nucleus and cytoplasm of cells (5).

This paper shows that the nuclear import of drug–PR complexes is a time- and dose-dependent process, saturable at 12.5 nM progesterone. It is rational that transport across the NPC is a saturable process for large cargo (PR is ~120 kd). The NPC does allow passive diffusion of molecules less than 45–60 kd. However, during active/facilitated transport, much larger molecules can enter, presumably because of changes in the NPC by import proteins. Indeed, some very huge molecules can get across the NPC with the assistance of their corresponding import receptors. Import of large cargo (60–125 kd) occurs not only with an import receptor (importin) but with Ran and a nonhydrolyzable version of GTP. Even larger cargo (500–669 kd) requires importin, Ran, and hydrolyzable GTP (8).

Interestingly, import of progesterone–PR complexes correlates well with transcriptional activity at low doses of progesterone. At higher doses, however, import does not continue to increase, even though transcriptional activity does. This can be explained as follows: transcriptional activity depends on at least two factors: (a) import of PR–progesterone complexes into the nucleus and (b) progesterone binding to PR already in the nucleus; the combination of these two factors occurs in receptors that are transcriptionally competent. At low doses of progesterone, import of PR–progesterone complexes into the nucleus and progesterone binding to PR already in the nucleus both may contribute to transcriptional activity. Because the dose is "low," the relative contribution of each mechanism to the increased transcriptional activity may be equal to or primarily driven by PR trafficking. At high doses of progesterone, transcriptional activation is governed by progesterone itself passively diffusing across the NPC and binding to PR already in the nucleus and less influenced by the import of progesterone–PR complexes into the nucleus

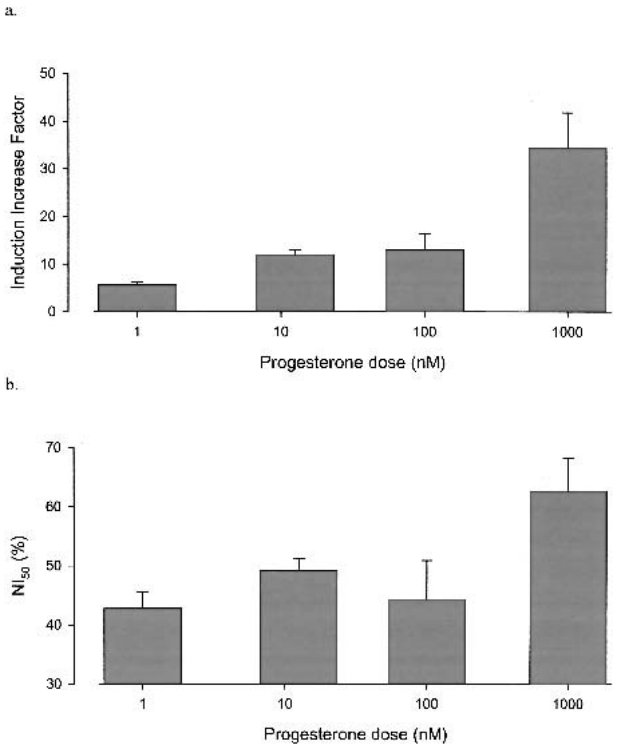

Fig. 7. Sigmoid model parameters are shown with standard error bars. (a) Induction increase factor vs. progesterone dose. (b) NI_{50} vs. progesterone dose.

because this process is saturated. Given that PRB is significantly nuclear even before the addition of drug, high doses of progesterone can activate these unoccupied receptors in the nucleus, leading to higher transactivation. Therefore, we postulate that the dose-dependent activation of genes (reporter gene) by PR depends on the presence of activated (drug-occupied) receptors in the nucleus, which can result from their import from the cytoplasm or from direct binding to receptors in the nucleus when there are high amounts of drug (when the dose is high).

Mager *et al.* (10) have also shown that GR binding to glucocorticoid response elements and residence time in the nucleus are key factors that govern transcriptional activity. Their paper (10) presents a quantitative structure–property relationship model for the *in vivo* transcriptional activity of several corticosteroids. Building on a series of refinements that couple *in vitro* data (like that collected here) with *in vivo* data collected from studies in rats, their modeling of corticosteroid effects has shown that gene-mediated effects are dependent on initial steroid levels, steroid dose, and the transit times, which affect how long the drug/receptor complex exists. The model presented here is a simplified version of the *in vitro* components of their model but lacks the *in vivo* model components. Nonetheless, it will be interesting to explore the relationship between transcriptional activity measured *in vitro* using fluorescence microscopy vs. levels measured *in vivo* in animal studies. This will be the focus of future work.

Likewise, work by Schaaf and Cidlowski (9) has shown that mobility of the glucocorticoid receptor (GR), once in the nucleus, is reduced with increasing dose of agonist, which allows more time for the complexes to interact with transcriptional machinery and other factors in the cell, leading to an increase in transactivation of target genes. This is likely to be the case for PR as well; increasing dose of progesterone should lead to decreased mobility of PR in the nucleus, also leading to higher transactivation. The glucocorticoid receptor is mostly cytoplasmic in the absence of drug (dexamethasone). Schaaf and Cidlowski did not measure the rate of import of GR into the nucleus (and therefore did not correlate import rate with dose). For a cytoplasmic receptor such as GR, transactivation may be a simple two-step process requiring import of drug–GR complexes into the nucleus followed by a decrease in mobility of drug–GR complexes once inside the nucleus (allowing more time for drug-occupied PRB to interact with transcriptional machinery). For PR, transactivation at low doses of progesterone may follow this simple two-step process. At higher doses of progesterone, however, activation of receptors already in the nucleus must also be considered.

ACKNOWLEDGMENTS

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APPENDIX

Assumptions

We make the following three basic assumptions: (a) because progesterone is a small molecule and lipophilic, it will passively diffuse into the cells without a time lag; (b) the concentration of the hormone in the cytoplasm will be constant; and (c) the total fluorescence of the whole cell does not change with time.

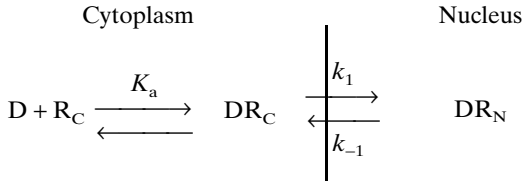
The cytoplasm fluorescence intensities from the different doses of progesterone were separately fitted for pharmacokinetic analysis. The fluorescence intensity vs. time (t) data can be described by:

$$C\% = Ae^{\lambda t} + B$$

C% is the percentage of total cytoplasmic intensity; λ is the rate constant that describes the rate of PR trafficking for each dose, in min^{-1}; and A and B are intercept coefficients.

Note that this model is a subset and simplified version of the model for the glucocorticoid receptor presented by Mager et al. (10).

For the simplest case:



where [D] is the hormone concentration in cytoplasm, $[R_C]$ is the cytoplasmic progesterone receptor concentration, $[DR_C]$ is the fluorescence intensity of hormone–progesterone receptor complex in the cytoplasm, $[DR_N]$ is the fluorescence intensity of hormone–progesterone receptor complex in the nucleus; K_a is the association constant of hormone and progesterone receptor, k_1 is the import rate constant of hormone–progesterone receptor complex, and k_{-1} is the export rate constant of hormone–progesterone receptor complex.

Derivation of the Equation

$$\text{Define } K_a = \frac{[DR_C]}{[D][R_c]}; \tag{1}$$

$$T = [R_C] + [DR_C], \tag{2}$$

$[R_C] = T - [DR_C]$; substitute into equation (1)

$$K_a = \frac{[DR_C]}{[D][T - [DR_C])}$$

$$K_a[D](T - [DR_C]) = [DR_C]$$

$$\text{So, } [DR_C] = \frac{K_a[D]T}{1 + K_a[D]}$$

The cytoplasm fluorescence intensity ($[DR_C]$) vs. time (t) profile can be described by a one-compartment model.

$$\frac{d[DR_C]}{dt} = -k_1[DR_C] + k_{-1}[DR_N] \tag{3}$$

$$\frac{d[DR_N]}{dt} = -k_{-1}[DR_N] + k_1[DR_C] \tag{4}$$

LaPlace transform equations (3) and (4), and solve for $[DR_c]$:

$$[DR_C] = \frac{k_{-1}[DR_C]_0 + k_{-1}[DR_N]_0}{k_1 + k_{-1}} + \frac{k_1[DR_C]_0 - k_{-1}[DR_N]_0}{k_1 + k_{-1}} e^{-(k_1+k_{-1})t}$$

It is already known that $[DR_C] = \frac{K_a[D]T}{1 + K_a[D]}$

SO,

$$[DR_C] = \frac{K_a[D]C\%T_0}{1 + [D]K_a} = \frac{k_{-1}[DR_C]_0 + k_{-1}[DR_N]_0}{k_1 + k_{-1}} + \frac{k_1[DR_C]_0 - k_{-1}[DR_N]_0}{k_1 + k_{-1}} e^{-(k_1+k_{-1})t}$$

$$C\% = \frac{1 + [D]K_a}{[D]K_aT_0}\left(\frac{k_{-1}[DR_C]_0 + k_{-1}[DR_N]_0}{k_1 + k_{-1}}\right) + \frac{1 + [D]K_a}{[D]K_aT_0}\left(\frac{k_1[DR_C]_0 - k_{-1}[DR_N]_0}{k_1 + k_{-1}}\right)e^{-(k_1+k_{-1})t}$$

$[DR_C]_0 = \frac{[D]K_aT_0}{1 + [D]K_a}$; $k_1 + k_{-1}$ is the slope of the curve (min^{-1})

T_0 is the total cytoplasm fluorescence intensity at time zero; and $[DR_N]_0$ is total nuclear fluorescence intensity of hormone–progesterone receptor complex at time zero.

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