Characterization of R5020 and RU486 Binding to Progesterone Receptor from Calf Uterus†

Cliff Hurd and Virinder K. Moudgil*
Department of Biological Sciences, Oakland University, Rochester, Michigan 48309-4401
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ABSTRACT: We have examined and compared the binding characteristics of the progesterone agonist R5020 [promegestone, 17,21-dimethylpregna-4,9(10)-diene-3,20-dione] and the progesterone antagonist RU486 [mifepristone, 17β-hydroxy-11β-[4-(dimethylamino)phenyl]-17α-(prop-1-ynyl)-estra-4,9-dien-3-one] in calf uterine cytosol. Both steroids bound cytosol macromolecule(s) with high affinity, exhibiting K_d values of 5.6 and 3.6 nM for R5020 and RU486 binding, respectively. The binding of the steroids to the macromolecule(s) was rapid at 4 °C, showing saturation of binding sites at 1–2 h for [^{3}H]progesterone and 2–4 h for both [^{3}H]R5020 and [^{3}H]RU486. Addition of molybdate and glycerol to cytosol increased the extent of [^{3}H]R5020 binding. The extent of [^{3}H]RU486 binding remained unchanged in the presence of molybdate, whereas glycerol had an inhibitory effect. Molybdate alone or in combination with glycerol stabilized the [^{3}H]R5020– and [^{3}H]RU486–receptor complexes at 37 °C. Although the rate of association of [^{3}H]RU486 with the cytosolic macromolecule was slower than that of [^{3}H]R5020, its dissociation from the ligand–macromolecule complex was significantly slower than [^{3}H]R5020. Competitive steroid binding analysis revealed that [^{3}H]progesterone, [^{3}H]R5020, and [^{3}H]RU486 compete for the same site(s) in the uterine cytosol, suggesting that all three bind to the progesterone receptor (PR). Sedimentation rate analysis showed that both steroids were bound to a molecule that sediments in the 8S region. The 8S [^{3}H]R5020 and [^{3}H]RU486 peaks were abolished by excess radioinert progesterone, RU486, or R5020. The results of this study suggest that, although there are some differences in the nature of their interaction with the PR, both R5020 and RU486 bind to the same 8S receptor in calf uterine cytosol.

Progesterone, a steroid hormone, is secreted by the corpus luteum in nonpregnant females. Its continued release from the corpus luteum and the placenta during pregnancy is crucial to the maintenance of the pregnant state. Upon its release, the hormone reaches to and affects the functioning of its major target tissues: the uterus, breast, and brain. Although the physiological manifestations of hormonal responses are quite well documented, the extact mode of steroid hormone action at the molecular level is still unclear. It is generally believed that upon entry into target cells, steroid hormones interact with intracellular receptors, forming complexes which subsequently undergo transformation to a form which possesses high affinity for nuclear sites. Molecules such as RU486[1] that appear to alter this chain of events are useful as tools for elucidating the molecular basis of hormone action and may be important as a possible means by which specific therapies could be instituted during hormone-related abnormalities (Philibert, 1984; Moudgil, 1985).

Specific hormonal antagonists can be used to investigate the molecular basis of hormone action (Crowley, 1986). RU486 is a synthetic molecule that possesses strong antiprogestational properties (Philibert, 1984). The clinical applications of this compound have already met with wide publicity (Couzinet et al., 1986). Although it has been hypothesized that RU486 may exert its effects by coupling with the PR in target tissues (Philibert, 1984; Couzinet et al., 1986), the precise nature of this molecular interaction, and how it may affect normal cellular functions, is not known. One clue to its mode of action is suggested by a recent report from this laboratory (Moudgil & Hurd, 1987) that demonstrated that the ability of PR to undergo 8S to 4S transformation is compromized significantly when it is bound to RU486. In order to examine whether the differences between the ability of RU486- and R5020-bound PR to transform were due to a distinct interaction of these steroids with receptor, a detailed analysis of steroid binding characteristics of calf uterne PR was undertaken in this study. Studies on the binding characteristics of RU486 are of great importance to understanding steroid hormone action, in particular, and to understanding contemporary pharmacology and molecular endocrinology, in general.

Materials and Methods

Chemicals. All reagents were of analytical grade and were prepared in deionized water. All procedures were carried out at 0–4 °C unless indicated otherwise. [^{3}H]Progesterone (40–50 Ci/mmol), [^{3}H]R5020 (70–87 Ci/mmol), and R5020 were purchased from New England Nuclear (Boston, MA). [^{3}H]RU486 (mifepristone, 30–35 Ci/mmol) and radioinert RU486 were gifts from Roussel Uclaf (Romainville, France). ATP, progesterone, cortisol, Na_2MoO_4, glycerol, EDTA, monothioglycerol, and charcoal were from Sigma Chemical Co. (St. Louis, MO). Ultrapure Tris was obtained from Schwarz/Mann (Cambridge, MA).

Buffers. The following buffers were prepared at 23 °C and were stored at 4 °C prior to use: buffer A, 20 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, and 8% glycerol, pH 7.5 at 23 °C; buffer B, 20 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, 8% glycerol, and 20 mM Na_2MoO_4, pH 7.5 at 23 °C; buffer C, 20 mM Tris-HCl, 1 mM EDTA, and 12 mM monothioglycerol, pH 7.5 at 23 °C; buffer D, 20

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* Correspondence should be addressed to this author.

[1] Abbreviations: PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetate; Pr, progesterone; PR, progesterone receptor; PRc, progesterone–receptor complexes; R5020, 17,21-dimethylpregna-4,9(10)-diene-3,20-dione; RU486, 17β-hydroxy-11β-[4-(dimethylamino)phenyl]-17α-(prop-1-ynyl)-estra-4,9-dien-3-one; Tris, tris(hydroxymethyl)aminomethane; GR, glucocorticoid receptor.

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mM Tris-HCl and 1 mM EDTA, pH 7.5 at 23 °C.

Preparation of Calf Uterine Cytosol. Calf uteri were obtained from a local meat processing company. The calves were sacrificed approximately 10 min before the uteri were removed. After removal, the tissue was packed in ice, transported to the lab, excised, rinsed in cold 0.9% NaCl, quick-frozen in precooled (−70 °C) ethanol, and stored at −70 °C until used. To prepare cytosol, the tissue was minced, rinsed with cold 0.9% NaCl, and homogenized in 4 volumes (v/w) of buffer A, B, or D with four 5–10-s bursts with a tissumizer (Tekmar, Model SDT). PMSF was added to a final concentration of 0.3 mM just after the first homogenization burst. The homogenate was centrifuged for 10 min at 10000*g*; the supernatant was collected and centrifuged 1 h at 150000*g*. The resultant cytosol was obtained by aspirating with a Pasteur pipet between the surface lipid layer and the pellet.

Steroid Binding Measurements. For quantitation of steroid–receptor complexes, portions of cytosol containing PR complexes were incubated for 5–10 min at 0 °C with an equal volume (v/v) of dextran-coated charcoal suspension (0.5% activated charcoal and 0.05% dextran in buffer D). Charcoal suspension and cytosol mixtures were centrifuged 5 min at 1000*g*; supernatants were removed and placed in scintillation vials for measurement of radioactivity.

Sedimentation Rate Analysis. Continuous 8–30% linear glycerol gradients (4.4 mL) were prepared in buffer B containing 0.01 M KCl using a Beckman gradient former (Beckman, Palo Alto, CA) (Moudgil, 1985; Moudgil et al., 1985, 1987). The gradients were kept at 4 °C for 1 h before use. Just prior to layering samples onto the gradients, aliquots (0.2 mL) containing PRc samples were incubated for 5–10 min with a charcoal pellet formed by sedimenting an equal volume of suspension (0.5% activated charcoal and 0.05% dextran in buffer D). Gradients were then centrifuged at 270000*g* in a Sorval TV-865 vertical rotor (Ivan Sorvall, Inc., Norwalk, CT) for 2 h, 15 min. At the end of the run, the gradient tubes were pierced at the bottom, and 21–23 fractions (0.2 mL) were collected into scintillation vials for measurement of radioactivity.

Saturation Binding Analysis. Cytosol was prepared as stated above in buffer B. Aliquots of cytosol in duplicate were incubated 4 h at 4 °C with different concentrations (0.5 nM–0.1 μM) of [^{3}H]R5020 or [^{3}H]RU486. Parallel cytosol samples were incubated with a 100-fold excess of radioinert R5020 or RU486 in the presence of [^{3}H]steroid in order to monitor nonspecific binding. Specific binding was calculated by subtracting the nonspecific amount of [^{3}H]steroid bound for a given concentration from the total bound at the same concentration. The extent of [^{3}H]steroid binding was determined by employing the charcoal adsorption assay as described earlier under steroid binding measurements.

Specificity of Steroid Binding. Cytosol was prepared in buffer B. Aliquots of cytosol were incubated with 0.1–20 μM concentrations of radioinert progesterone, R5020, RU486, or cortisol. These mixtures were incubated 10 min at 0 °C prior to the addition of [^{3}H]R5020 or [^{3}H]RU486 to a 20 nM concentration. After a 4-h incubation period at 0 °C with [^{3}H]steroid, the extent of [^{3}H]steroid binding was determined by performing charcoal adsorption assays.

Effects of ATP, Molybdate, and Glycerol on the Binding of [^{3}H]R5020 and [^{3}H]RU486 to Cytosol PR. Cytosol was prepared in buffer C plus 3 μM cortisol. Aliquots were incubated with 1–10 mM ATP, 1–20 mM Na_2MoO_4, or 1–20% glycerol for 30 min at 4 °C. Portions were then incubated with 20 nM [^{3}H]R5020 or [^{3}H]RU486 for 4 h at 4 °C. Charcoal adsorption assays were then performed to determine the extent of ^{3}H ligand binding.

Analysis of the Effects of Molybdate and Glycerol on the Stability of PR Complexes at 37 °C. Cytosol was prepared in buffer C plus 3 μM cortisol. Samples were divided and incubated for 2 h at 4 °C with [^{3}H]R5020 or [^{3}H]RU486 to form complexes. Aliquots of cytosol were incubated 5 min at 4 °C with 20 mM Na_2MoO_4, 20 mM Na_2MoO_4 plus 20% glycerol, or 20% glycerol alone and then incubated at 37 °C heat-block for up to 60 min. Portions (0.2 mL) were removed from heat treatment at 0-, 5-, 30-, and 60-min time intervals and charcoal-assayed to determine the extent of [^{3}H]steroid binding.

Effects of Molybdate and Glycerol on the Rate of Exchange of Steroid at 37 °C. Cytosol was prepared in buffer B and incubated with 20 nM [^{3}H]R5020, [^{3}H]progesterone, or [^{3}H]RU486 for 18 h at 4 °C. A 100-fold excess of radioinert R5020 was then added to each sample. Samples were incubated at 37 °C, 0.1-mL samples were removed at 0-, 5-, 10-, and 20-min time intervals and treated with charcoal in order to determine the amount of bound [^{3}H]steroid.

Analysis of Specific Binding during Heat Treatment. Cytosol was prepared in buffer A plus 3 μM cortisol. Aliquots of cytosol were incubated for 2 h at 4 °C with 20 nM [^{3}H]-progesterone or [^{3}H]RU486. Parallel samples were incubated with 100-fold excess radioinert progesterone prior to the addition of [^{3}H]progesterone or [^{3}H]RU486. Aliquots of cytosol were then removed from ice after 0-, 30-, 60-, and 120-min time intervals. All samples were then treated with charcoal to determine the extent of steroid binding. Nonspecific binding was subtracted from the total binding to determine the amount of specifically bound [^{3}H]steroid.

Results

Previous studies have shown that for quantitative analysis of PR in vitro, use of R5020 (a synthetic progestin) has certain advantages over the use of progesterone, R5020 has a higher affinity for PR and a slower dissociation rate from the receptor–ligand complex, and it is not bound by corticosteroid binding globulin (Philibert et al., 1977; Haslam & Shyamala, 1979). It was also shown to be a very potent competitor for [^{3}H]progesterone binding sites in the calf uterine cytosol (Theofan & Notides, 1984). For these reasons, we have used R5020 as a ligand for studying PR.

Figure 1 shows that the binding curves were similar when calf uterine cytosol was incubated with increasing concentrations of R5020 or RU486. Both steroids saturated specific sites at concentrations between 10 and 20 nM. Scatchard analysis of the steroid binding data showed a linear plot, indicating the presence of a single class of binding sites. If differences in specific activity between the ligands are taken into consideration, the number of R5020 binding sites does not appear to be higher, as might otherwise be suggested. The K_d values for both R5020 (~5.6 nM) and RU486 (~3.6 nM) are similar and show high affinity of each ligand for the binding site(s).

The rate of association of progesterone, R5020, and RU486 with the macromolecule(s) is shown in Figure 2. All three steroids exhibited similar rates of association, rapidly reaching an equilibrium binding state at a 20 nM concentration. For both RU486 and R5020, maximum binding was reached after a 4-h incubation at 0 °C. Progesterone appeared to reach a saturation plateau more rapidly than either RU486 or R5020. These data also show that progesterone–, R5020–, and RU486–receptor complexes remained stable for up to 20 h at 4 °C in the presence of molybdate.

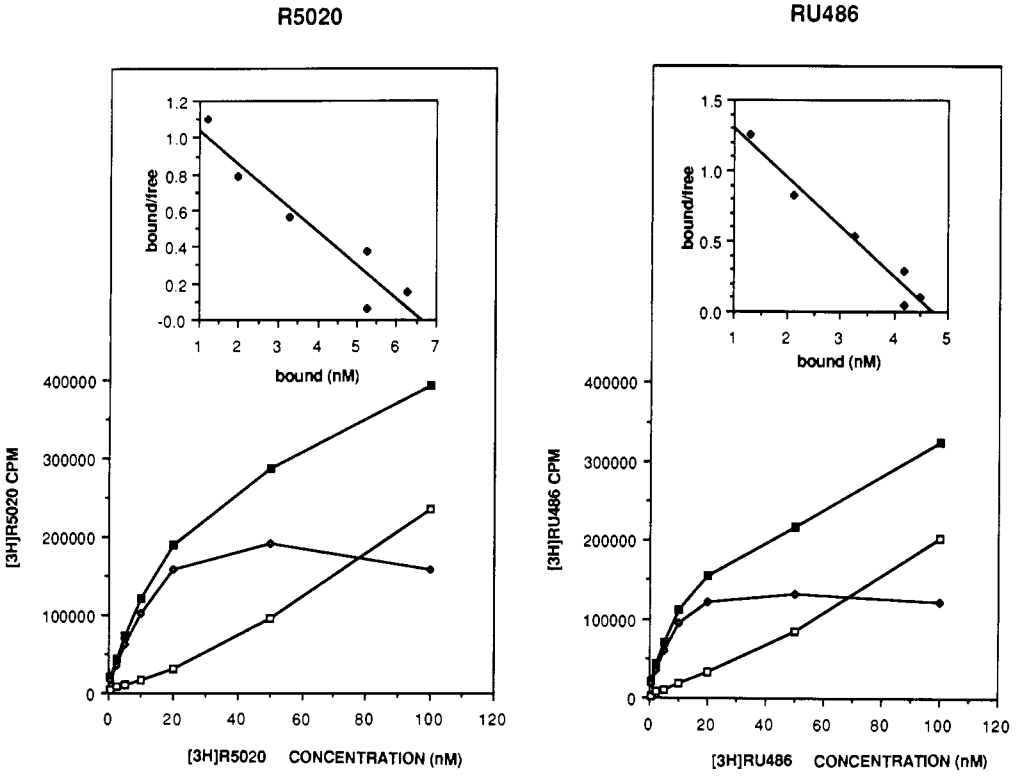

FIGURE 1: Saturation binding analysis. Calf uterine cytosol was prepared in buffer B, and 100-μL aliquots were incubated for 4 h at 0 °C with varying concentrations (0.5 nM–0.1 μM) of [^{3}H]R5020 (left) or [^{3}H]RU486 (right). Nonspecific steroid binding (□) was determined by preincubating parallel samples with a 100-fold excess of radioinert steroids. Charcoal assay was employed to determine the extent of macromolecule-bound radioactivity. Specific steroid binding (◊) was calculated by subtracting nonspecific from total (■) steroid binding. Inset: Scatchard analysis. R5020 binding, K_D = 5.6 nM; RU486 binding, K_D = 3.6 nM.

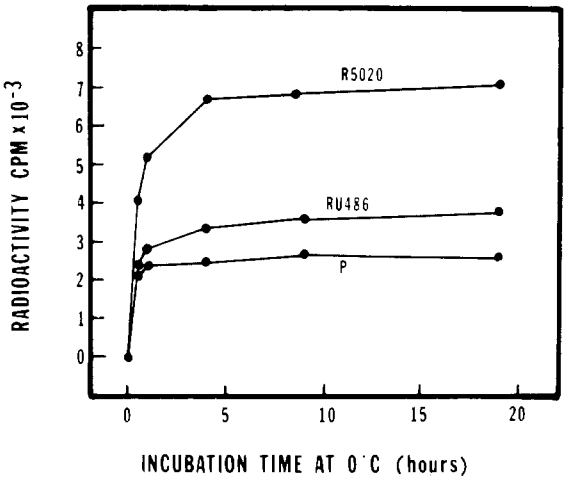

FIGURE 2: Equilibrium binding analysis of [^{3}H]R5020, [^{3}H]progesterone, and [^{3}H]RU486 in calf uterine cytosol. Cytosol was prepared in buffer B plus 3 μM cortisol and brought to a 20 nM concentration with respect to [^{3}H]progesterone, [^{3}H]R5020, or [^{3}H]RU486. Samples (0.2 mL) were removed at appropriate time intervals and treated with an equal volume of charcoal–dextran suspension to remove free steroids. The specific steroid binding was determined as described in the legend to Figure 1.

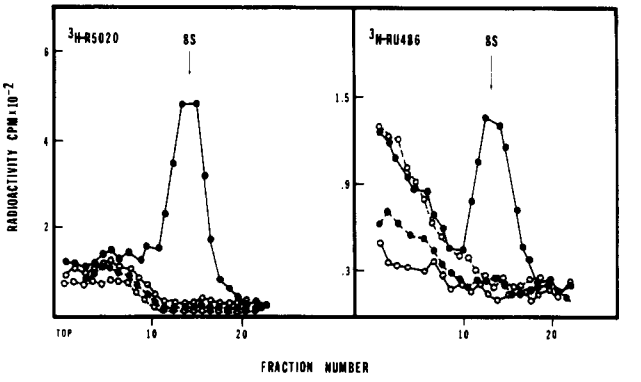

FIGURE 3: Specificity of 8S and 4S PR forms bound to [^{3}H]R5020 or [^{3}H]RU486. Cytosol was prepared in buffer A and incubated 2 h at 4 °C with (left panel) 20 nM [^{3}H]R5020 (●—●) or with [^{3}H]R5020 plus 100-fold excess of radioinert progesterone (○—○), RU486 (●---●), or R5020 (○---○), or (right panel) 20 nM [^{3}H]-RU486 (●—●) or with [^{3}H]RU486 plus 100-fold excess radioinert progesterone (○—○), RU486 (●---●), or R5020 (○-¬-○). Samples (0.2 mL) were layered on 8–30% linear glycerol gradients and centrifuged in a vertical rotor at 270000g for 2 h, 15 min. The rate of sedimentation of the receptor was determined by using a standard curve and by comparison with the sedimentation position of the internal markers (glucose oxidase, 7.9 S; peroxidase, 3.6 S).

The anti-progestin RU486 and the progestins R5020 and progesterone appeared to compete for the same binding site in calf uterine cytosol (not shown). Full displacement of [^{3}H]RU486 was accomplished when cytosol was incubated with a 10-fold excess of either radioinert progesterone or RU486. Likewise, [^{3}H]R5020 was displaceable by a 10-fold excess of radioinert RU486 or R5020.

Sedimentation rate analysis (Figure 3) demonstrated that PR migrated as an 8S moiety in 8–30% linear glycerol gradients whether it was complexed with [^{3}H]R5020 or [^{3}H]-RU486. The 8S peak of radioactivity has been shown to represent the nonactivated form of the receptor (Wolfson et al., 1980; Loosfelt et al., 1984; Moudgil, 1985; Moudgil et al., 1985, 1987; Singh & Moudgil, 1985). Binding of both [^{3}H]R5020 and [^{3}H]RU486 was displaced by an excess of radioinert progesterone, R5020, or RU486, suggesting that all three steroids are interacting with the same site(s) on the receptor.

Many chemical agents have been reported to influence the hormone binding properties of steroid receptors, including molybdate (Nishigori & Toft, 1980; Wolfson et al., 1980; Bevin & Bashirelahi, 1980; Tong et al., 1981; Loosfelt et al.,

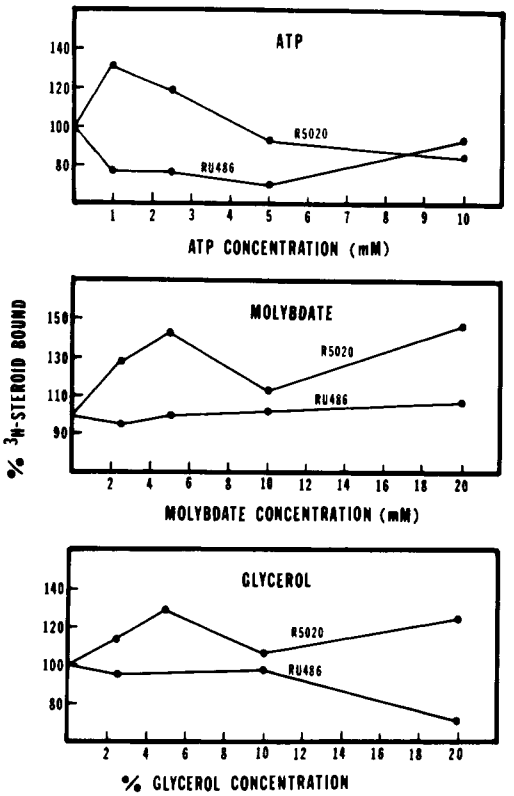

FIGURE 4: Effects of ATP, molybdate, and glycerol on the binding of [^{3}H]R5020 and [^{3}H]RU486 to cytosol PR. Cytosol was prepared in buffer C plus 3 μM cortisol. Aliquots of cytosol were incubated with 1–10 mM ATP (top), 1–20 mM Na_2MoO_4 (middle), or 1–20% glycerol (bottom) for 30 min at 4 °C. Portions were then incubated with 20 nM [^{3}H]R5020 or [^{3}H]RU486 for 4 h at 4 °C. Charcoal adsorption assays were performed to determine the amount of ^{3}H-labeled ligand–receptor complexes.

1984; Moudgil, 1985), glycerol (Feil et al., 1972; Ogle, 1980), and ATP (Sando et al., 1979a,b; Moudgil & John, 1980). In this study, we tested the effects of these agents on the binding of R5020 and RU486 to calf uterine PR (Figure 4). The indicated values represent the mean of duplicate samples. Low concentrations of ATP (1 mM) appeared to enhance the binding of R5020 to the PR (Figure 4). However, when the concentration of ATP was raised above 5 mM, there was a decrease in the extent of this binding. Low concentrations of ATP (1–5 mM) decreased the amount of RU486 binding, but higher concentrations (above 5 mM) returned the level of RU486 binding to that seen in the control. Both gycerol (bottom panel) and molybdate (middle panel) enhanced the binding of R5020 to PR. Molybdate had little effect on the extent of RU486 binding while glycerol appeared to decrease the extent of RU486 binding especially as its concentration approached 20%.

We have also examined the effects of molybdate and glycerol on the stabilization of the R5020– and RU486–receptor complexes at 37 °C. The results in Figure 5 show that molybdate alone or in combination with glycerol stabilized the steroid–receptor complexes. Glycerol alone did not appear to significantly affect the stability of the hormone–receptor complexes. Heat treatment at 37 °C dissociated complexes equally well in the presence or absence of glycerol.

In order to better understand the binding kinetics of RU486, the dissociation rate of the RU486–receptor complex was compared to that of the PR and the R5020–receptor complexes. Since molybdate stabilized the PR at 37 °C (Figure 5), it was possible to include molybdate in the homogenization buffer, in order to stabilize the receptor, and examine dissociation rates by exchanging radioligand for radioinert ligand at 37 °C. The rate of dissociation or exchange of progesterone from its receptor was the most rapid, followed by the dissociation of R5020 and RU486 (Figure 6). After 5 min, [^{3}H]PR complexes had fully dissociated. At this same time period, [^{3}H]RU486–receptor complexes showed only 50% dissociation. After 20 min, [^{3}H]R5020–receptor and [^{3}H]PR complexes had fully dissociated; however, 20% of [^{3}H]-RU486–receptor complexes remained intact. The slower dissociation rate of RU486 from the calf uterine PR reported here supports the findings of Philibert (1984), who showed a higher affinity and slower dissociation rate of RU486, than R5020, from the rat uterine progesterone receptor.

Since we had employed thermal activation of PR at 23 °C (Hurd & Moudgil, 1986; Moudgil & Hurd, 1987), it was important to determine the influence of elevated temperature

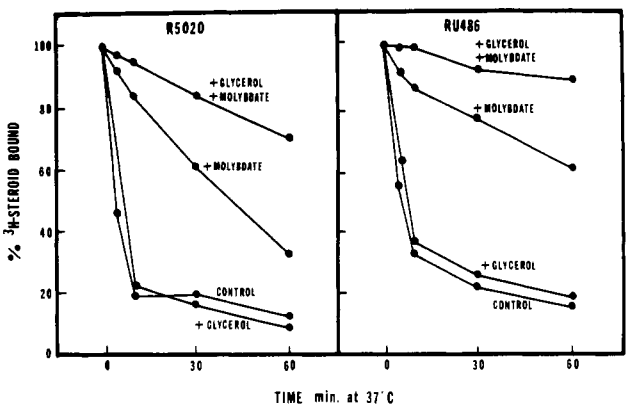

FIGURE 5: Analysis of the effects of molybdate and glycerol on the stability of PR complexes at 37 °C. Cytosol was prepared in buffer C plus 3 μM cortisol. Steroid–receptor complexes were formed by incubating cytosol with 20 nM [^{3}H]R5020 or [^{3}H]RU486 for 4 h at 0 °C. Aliquots of cytosol were incubated for 30 min at 4 °C with 20% glycerol, with 20 mM Na_2MoO_4, or with 20% glycerol plus 20 mM Na_2MoO_4. Samples were subsequently incubated at 37 °C for 0–60 min. Portions (0.2 mL) were removed and brought to 0 °C at the times indicated. These samples were charcoal-assayed to determine the extent of [^{3}H]steroid binding.

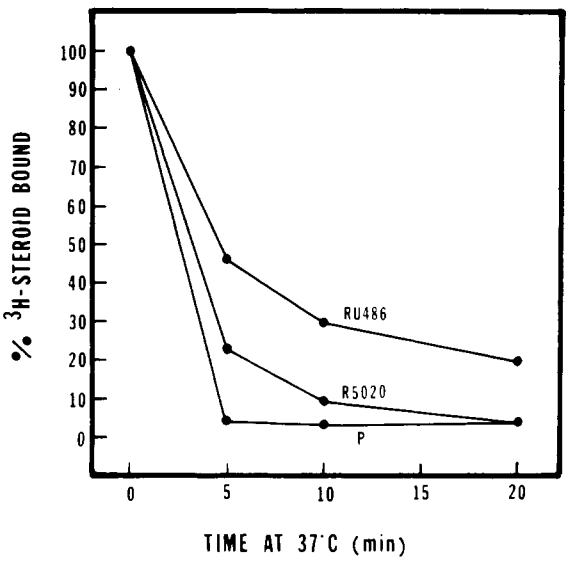

FIGURE 6: Influence of temperature on the rate of exchange of steroids at 37 °C. Cytosol was prepared in buffer B and incubated with 20 nM [^{3}H]R5020, [^{3}H]progesterone, or [^{3}H]RU486 for 18 h at 4 °C. A 100-fold excess of radioinert progesterone was then added to each sample. Samples were then transferred to a temperature block (37 °C), and 0.1-mL samples were replaced in ice at 5-, 10-, and 20-min time intervals. The extent of steroid binding was determined by employing the charcoal adsorption assays.

on the stability of the steroid–receptor complexes. Results obtained with experiments examining the effect of the 23 °C incubation on the population of specifically bound progesterone and RU486–receptor complexes showed that the rate of loss of progesterone–receptor complexes was greater than RU486–receptor complexes (not shown). The greatest difference in the binding of these two ligands appeared after heat treatment for 1 h; the total number of RU486–receptor complexes had declined approximately 25%; the amount of progesterone–receptor complexes had dropped by 50%.

Discussion

Hormones showing a strong affinity for their specific receptors are known to display agonist activity (Philibert et al., 1977; Haslam & Shyamala, 1979; Theofan & Notides, 1984). Before the effects of RU486 were known, all compounds with high relative binding affinity for the PR were also progestin agonists (Philibert et al., 1977; Philibert, 1984). However, despite the high relative binding affinity of RU486 for PR, it shows potent anti-progestin activity both in vivo and in vitro (Philibert, 1984). It is, therefore, clear that steroid-induced biological activity is not determined by binding alone. This unique characteristic of RU486 makes understanding its mode of action both intriguing and an important topic in the molecular biology of reproduction. Although antihormonal effects of RU486 have been observed in many mammalian species, its antagonist action in cow has not been reported. In order to better understand the nature of the molecular interaction between RU486 and the mammalian PR, we compared the binding characteristics of RU486 and R5020 in calf uterine cytosol.

Calf uterus is a rich source of unoccupied PR, and sufficient quantities of the latter can be obtained for biochemical analyses without pretreating the animal with estrogen (Theofan & Notides, 1984). Although the RU486 binder in calf uterus appears to have attributes of PR in general, our recent work has shown that the ability of RU486–receptor complexes to transform from an 8S to 4S form may be impaired (Hurd & Moudgil, 1986; Moudgil & Hurd, 1987).

While both RU486 and progesterone have been shown to bind PR with comparable affinity, RU486 binds to GR with a higher affinity than either dexamethasone or triamcinolone acetonide (Philibert, 1984; Bourgeois et al., 1984; Jung-Testa & Baulieu, 1984; Groyer et al., 1985). Consistent with these observations, results of our studies show that RU486 binds to calf uterine PR in a saturable and specific manner. Furthermore, this binding was cross-competable with R5020, progesterone, and RU486. The nonactivated, R5020– and RU486–receptor complexes sedimented in the 8S region with the radioactivity peaks in both cases displaceable by radioinert progestin or RU486.

Both R5020 and RU486 saturate specific sites at concentrations between 10 and 20 nM. Scatchard analysis of binding data resulted in a linear plot, demonstrating the presence of a single class of high-affinity binding sites. Results of earlier studies had suggested that binding of progesterone to its receptor might involve a cooperative process as evidenced by a convex Scatchard plot of binding data (Theofan & Notides, 1984). In this study, the analysis was carried out with a greater number of points at low steroid concentrations, a difference which might have contributed to our inability to observe a convexity in the Scatchard plot.

Molybdate (Bevins & Bashirelahi, 1980; Nishigori & Toft, 1980; Moudgil, 1985) and glycerol (Feil et al., 1972; Ogle, 1980), consistent with the results of others, enhanced the binding of progestin to PR. ATP at low concentrations (1–3 mM) in this and other studies (Moudgil et al., 1985) was shown to enhance the binding of R5020 to PR. RU486 binding, however, was inhibited by low concentrations of ATP, and molybdate had no effect on the binding of RU486. Interestingly, high concentrations of glycerol (20%) inhibited the formation of RU486–receptor complexes, which conflicts with the general observations of glycerol effect on steroid binding to receptors (Feil et al., 1972; Ogle, 1980) but is consistent with a similar effect of glycerol noted by Gravanis et al. (1984) on the formation of human RU486–receptor complexes. In addition, at 37 °C, molybdate stabilized both RU486– and R5020–receptor complexes, and glycerol appeared to potentiate this effect.

The more rapid exchange of [^{3}H]Pr and [^{3}H]R5020 for radioinert Pr, when compared with RU486 (Figure 5), is probably due to a greater stability of PR when it is bound to RU486 than when it forms complexes with progestins. These results, therefore, show that although RU486 and R5020 bind to PR in calf uterine cytosol, there are some qualitative differences. Recent work from this laboratory has also shown that progesterone receptors from mammalian and avian sources have differential affinity for RU486 and that RU486 binds to a macromolecule in chick oviduct cytosol that is immunologically distinct from PR (Moudgil et al., 1986; Eliezer et al., 1987).

RU486 binds with high affinity to PR from many species and sources, in addition to its reported binding to glucocorticoid and androgen receptors (Philibert, 1984; Baulieu, 1985). The molecular mechanism of RU486 action as a contragestive agent is still unclear. RU486 appears to act reversibly at the molecular level of receptor binding, irreversibly interrupting target cell integrity which is dependent on continued steroid action (Baulieu, 1985). RU486 binds to both native (8S) and transformed (4S) rat liver glucocorticoid receptor, and RU486–receptor complexes exhibit DNA binding in vitro (Agarwal et al., 1985). In addition, RU486 appears to transform the receptor to which it binds; the resulting complexes have been reported to be detected in the nuclear fraction of homogenates (Baulieu, 1985). Although RU486–receptor complexes can undergo transformation, the process appears to be quantitatively compromized (Bourgeois et al., 1984; Agarwal et al., 1985; Hurd & Moudgil, 1986; Moudgil & Hurd, 1987). It is difficult to ascribe all the reported antagonist-like effects of RU486 in vivo to a "less efficient" transformation process observed in vitro, although this may result in a decreased binding of the antagonist–receptor complexes to nonspecific and high-affinity chromatin sites or DNA sequences (Baulieu, 1985). It is, therefore, very important to examine various parameters of steroid hormone–receptor dynamics in order to understand both the agonist and antagonist action of hormones and antihormones. The similarities and differences between the steroid binding properties of progesterone, R5020, and RU486 reported in this paper should aid in such endeavors.

Acknowledgments

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Registry No. R5020, 34184-77-5; RU486, 84371-65-3; ATP, 56-65-5; MoO_4^{2-}, 14259-85-9; progesterone, 57-83-0; glycerol, 56-81-5.

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