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Analysis of Chicken Progesterone Receptor Structure Using a Spontaneous Sheep Antibody†

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ABSTRACT: A spontaneous sheep antibody to chick progesterone receptor was characterized and used as a tool to study the receptor structure. The antibody, which is present to some extent in sera from about one-third of the sheep tested, binds to *Staphylococcus aureus* protein A–Sepharose and therefore appears to be an IgG. It is specific for the chick progesterone receptor and does not react with free progesterone or with any of the other proteins tested, including other receptors and corticosteroid binding globulin. The antibody is nonprecipitating and has a very low titer (equivalence point = 2.5 pmol of receptor/mL of serum). The interaction of the receptor with the antibody was measured, and an apparent dissociation constant of 2×10^{-9} M was determined from these studies. The antibody reacts equally well with the two receptor subunits A and B but does not appear to react with the native aggregate form found in the cytosol. Thus, the immunologic site is occluded in the aggregate, and therefore the antibody will be a useful probe for this important region of the proteins. The antibody recognition sites on the receptors were further characterized by analysis of a proteolytic digest of receptors by using an endogenous Ca^{2+}-activated neutral protease. Competition studies using native receptor and receptor digests demonstrated that the antigenic site was not destroyed in the digest and was separated from the hormone binding fragment. We conclude that receptor subunits A and B have a cross-reactive immunologic site on a portion of the molecule other than the hormone binding domain.

The hen oviduct is a target tissue for progesterone. Oviduct cytosol contains a receptor protein made up of the two hormone binding subunits A and B (M_r 79 000 and 106 000, respectively) (Vedeckis et al., 1978). The proteins have kinetically identical progesterone binding sites (Schrader & O'Malley, 1972; Hansen et al., 1976) but differ in their interactions with nuclear constituents (Schrader & O'Malley, 1972; Schrader et al., 1972, 1975). Proteolytic digestion studies (Vedeckis et al., 1980; Sherman et al., 1974, 1976; Sherman & Diaz, 1977) have established the structural similarity of the two proteins, but peptide mapping studies have indicated that they are separate gene products (N. L. Weigel, unpublished experiments). Until now, no antibodies to chick progesterone receptor have been available for analysis of this receptor. In order to analyze their structural and functional domains, we attempted to raise antibodies in sheep to purified progesterone receptor A and B subunits. The approach has been sucessfully undertaken to raise antibodies to calf uterine estrogen receptor (Greene et al., 1977). During these studies, we found that about one-third of the sheep tested had an endogenous immunoglobulin activity of low titer which reacts with the chick progesterone receptor. This activity is reminiscent of "5S-CA" activity described by Fox (1978) but differs in that this immunoglobulin recognizes cytoplasmic receptors. This paper describes the interaction of these sheep sera with the chick receptor subunits and with proteolytic fragments derived from the receptor.

Materials and Methods

[1,2-$^3H_2$]Progesterone (50 Ci/mmol) and [6,7-$^3H_2$(N)]-triamcinolone acetonide (37 Ci/mmol) were purchased from New England Nuclear Co. R5020, [17α-*methyl*-^{3}H]-17α,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (55.4 Ci/mmol), was from Roussel-UCLAF, Romaineville, France. Nonradioactive steriods were obtained from Steraloids.

All chemicals were reagent grade. Tris, ammonium sulfate, and sucrose were ultrapure grade from Schwarz/Mann. Ion-exchange resins were from Whatman. Poly(ethylene

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glycol) MW6000 was from Sigma, and *Staphylococcus aureus* protein A–Sepharose C1-4B was from Pharmacia. Freund's complete adjuvant was obtained from Difco and rabbit anti-sheep IgG from Miles.

All steps of the receptor purification procedure were performed in buffer A (0.01 M Tris-HCl containing 0.001 M Na_2EDTA and 0.012 M 1-thioglycerol, pH 7.4). To this buffer were added appropriate amounts of potassium chloride as indicated in the text.

Aqueous samples (500 μL) were counted in 4 mL of Amersham-Searle ACS scintillation cocktail in 7-mL vials with a Beckman LS-233 scintillation spectrometer. Counting efficiency was 33%.

Antigen. Purified A and B progesterone receptor subunits were prepared from hen oviducts by a modification of the procedures earlier described for purification of the A subunit from chick oviduct (Coty et al., 1979) and for purification of the B subunit from hen oviduct (Schrader et al., 1977). The B subunit was 50–80% of the protein, and the A subunit was 10–20% of the protein. The partially purified receptor was labeled with [^{3}H]progesterone (5 Ci/mmol) in order to follow the purification and estimate the amount of receptor complex used for immunization.

Immunization. One-year-old male sheep were castrated several months before starting the immunization. Each sheep was injected at multiple sites on the back subdermally with an emulsion prepared by homogenizing 1 mL of 0.9% NaCl solution (containing about 100 μg of receptor protein) with 1 mL of Freund's complete adjuvant. Booster injections prepared in the same manner were given at intervals of 1 month.

Blood was collected from the jugular vein and allowed to clot. Serum was separated from red blood cells by centrifugation and stored at −20 °C. For most experiments, a crude immunoglobulin fraction was prepared by adding saturated ammonium sulfate in buffer A to a final concentration of 35% saturation. The precipitate was collected by centrifugation at 13000*g* for 30 min, and the pellet was redissolved in half the original volume of buffer A containing 0.15 M KCl. After extensive dialysis against the same buffer, another centrifugation was performed at 13500*g* for 1 h, and the supernatant was used as a crude IgG fraction.

Hormone Receptor Complexes. Chick cytosol was prepared as previously described (Schrader & O'Malley, 1972), and the progesterone receptor was labeled by incubating the cytosol with [^{3}H]progesterone (1.5×10^{-8} M) for 2 h at 4 °C.

The receptor subunits were then dissociated and precipitated by addition of saturated $(NH_4)_2SO_4$ in buffer A to a final concentration of 30% saturation. The sample was centrifuged at 13200*g* for 30 min, and the pellet was redissolved in half the original volume of buffer A containing 0.15 M KCl and dialyzed against the same buffer. Aliquots of this material were used for immunochemical reactions as well as for further purification. Subunits A and B were separated by applying the receptor diluted 1:4 (v/v) with buffer A to a 1-mL DEAE-cellulose column equilibrated with buffer A. The column was washed with 6 mL of buffer A; the A protein eluted with buffer A containing 0.15 M KCl and the B protein with buffer A containing 0.3 M KCl, as described previously (Schrader & O'Malley, 1972).

Intact complex and monomer forms of the receptor were prepared by the procedure of Maggi et al. (1981) through the DEAE-cellulose step in order to compare directly the interactions of the monomers and complex with the IgG. Briefly, chick cytosol was prepared and labeled with [^{3}H]progesterone as usual. The receptor complex was precipitated with 10% poly(ethylene glycol) MW6000, resuspended, and passed through phosphocellulose and DNA columns which remove monomers. The receptor was then applied to a DEAE-cellulose column, washed with 0.15 M KCl in buffer A, and eluted with 0.3 M KCl in buffer A, and a portion was immediately diluted with buffer A to 0.15 M KCl to prevent dissociation of the complexes. Another portion was diluted with an equal volume of 0.3 M KCl in buffer A to produce monomers. The two samples were left overnight and used the following day.

Unlabeled meroreceptor digests were prepared for competition studies by incubating cytosol with 1.0×10^{-6} M estradiol for 2 h (which protects the progesterone binding site) followed by digestion with the endogenous Ca^{2+}-activated protease in buffer A containing 0.1 M $CaCl_2$ for 2 h. The Ca^{2+} was then removed and the estradiol replaced by progesterone by dialysis against buffer A containing 2×10^{-8} M progesterone. A portion of the digest was treated as described above but was dialyzed against 2×10^{-8} M [^{3}H]progesterone (50 Ci/mmol) in order to determine the extent of digestion and amount of meroreceptor formed. Both portions were then dialyzed against buffer A to remove free hormone. The labeled portion was counted and applied to a Sephadex G-100 column. All of the label was found in the meroreceptor peak (Vedeckis et al., 1980) or in the free hormone peak, so the protein was completely digested as expected. The digest labeled with cold progesterone was used in the competition study.

Other hormone receptor complexes were prepared in a similar manner. Chick liver cytosol was prepared as described for chick oviduct, the glucocorticoid receptor was labeled with [^{3}H]triamcinolone acetonide, and partial purification was performed as described for chick oviduct. Glucocorticoid receptor in chicken oviduct was studied by using the same cytosol as used for the progesterone receptor, with only the preparation labeled with triamcinolone acetonide.

[^{3}H]Estradiol receptor complex was prepared from uteri of female rats which had been ovariectomized at least 2 weeks prior to use. The uteri were homogenized by using a Duall all-glass homogenizer equipped with a motor-driven pestle in 0.01 M Tris-HCl, pH 7.4, containing 1.5 mM Na_4EDTA at room temperature. The homogenate was centrifuged at 800*g* for 10 min and the supernatant centrifuged at 39000*g* for 20 min. The resulting supernatant was adjusted to a fresh weight equivalent to 150 mg of tissue/mL of buffer with the above buffer and labeled with 20 nM [^{3}H]estradiol for 30 min at 37 °C. The receptor was precipitated with ammonium sulfate at 50% saturation and dialyzed against buffer A containing 0.15 M KCl.

Rabbit uterine progesterone receptor was prepared from virgin New Zealand white rabbits which were injected subcutaneously each day for 5 days with 100 mg of estradiol benzoate in sesame oil prior to use. The uteri were excised, and the endometrium was homogenized in buffer A containing 10% glycerol. The cytosol was then prepared and labeled as described for the chick receptor.

Human progesterone receptor was prepared from MCF-7 cells, a human breast tumor line containing progesterone receptors (Lippman et al., 1977). Cells grown in the presence of 10^{-9} M estradiol in order to stimulate production of progesterone receptor were harvested by incubation in Hanks EDTA for 10 min at 37 °C followed by centrifugation at low speed to pellet the cells. The cells were resuspended in buffer A containing 0.15 M KCl, homogenized, and centrifuged at 105000*g* for 30 min. The supernatant was incubated with 20

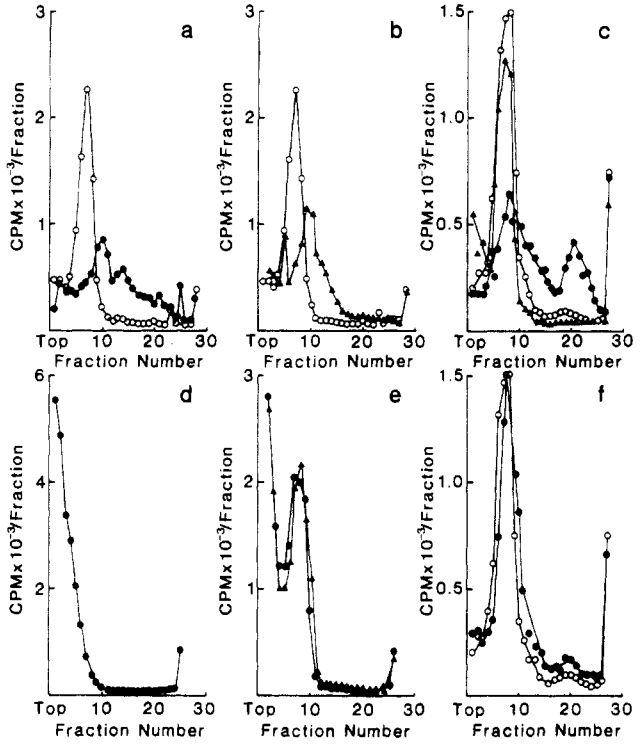

FIGURE 1: Sucrose-gradient analysis of interactions of crude sheep IgG. (a) Chick progesterone receptor (0.5 pmol) was incubated at 4 °C with 250 μL of crude IgG fraction and centrifuged for 20 h in sucrose gradients containing 0.15 M KCl: (○) receptor alone; (●) receptor + IgG from sheep R73 (an immunoreactive sheep). (b) Samples were prepared and analyzed as described in (a): (▲) receptor + IgG from a nonreactive sheep; (○) receptor alone. (c) Samples were prepared and analyzed as in (a), but 0.3 M KCl was included in the gradients: (○) receptor alone; (●) receptor + 50 μL of IgG from R73; (▲) receptor + 200 μL of IgG from nonreactive sheep. (d) [^{3}H]Progesterone was incubated with IgG from R73 and centrifuged as described in (a). (e) ^{3}H-Labeled corticosteroid binding globulin (CBG) was incubated with crude IgG and centrifuged for 16 h in gradients containing 0.15 M KCl without glycerol: (●) CBG + IgG from R54, an immunoreactive sheep; (▲) CBG + IgG from a nonreactive sheep. (f) Rabbit anti-sheep IgG, 0.4 mg, was incubated with 0.25 pmol of receptor and centrifuged for 20 h as described under Materials and Methods: (○) receptor alone; (●) receptor + IgG.

nM ^{3}H-labeled R5020 for 3 h on ice and treated with 0.5% charcoal and 0.05% dextran T60 to remove free hormone before use.

Chick serum was also labeled with [^{3}H]progesterone as a source of serum steroid binding proteins.

Immunochemical Assays. (*a*) *Sedimentation Studies.* Partly purified steroid receptor complexes (50–150 μL containing 0.5–2 pmol of [^{3}H]progesterone receptor) were incubated with 50–250 μL of crude IgG fraction for 8–16 h at 4 °C with continuous gentle agitation. Aliquots (250 μL) were layered on top of 5 mL of 5–25% (w/v) sucrose gradients in buffer A containing 10% glycerol and either 0.15 or 0.3 M KCl. After centrifugation was performed by using a Beckman SW50.1 rotor at 253000*g* for 16–20 h as indicated in the text, 200-μL fractions were collected, and radioactivity was measured. In selected cases, ^{14}C-labeled bovine serum albumin (4.4 S) was centrifuged in a separate gradient to serve as a sedimentation marker.

(*b*) *Protein A–Sepharose Assays.* The indicated amounts of receptor (usually 5–100 μL) and sheep IgG fractions were incubated in buffer A containing 0.15 M KCl for 4 h at 4 °C. The samples were applied to 0.2-mL *Staphylococcus aureus* protein A–Sepharose columns prepared in Pasteur pipets and washed once with 2 mL of buffer A and twice with 2 mL of buffer A containing 1 M KCl. The bound receptor was eluted with 2 mL of 1 N acetic acid and counted for ^{3}H. The columns were immediately washed with buffer A and reused. Omission of the second 2.0 mL of buffer A containing 1 M KCl resulted in a slight increase in background; additional acid elution did not release any more radioactivity.

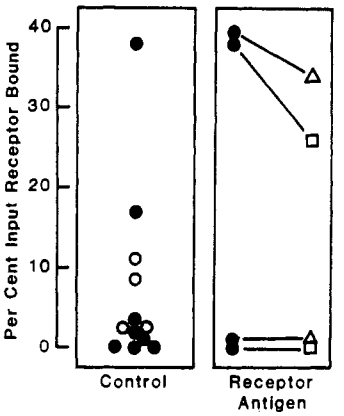

FIGURE 2: Occurrence of reactive antibody in sheep sera. Crude IgG (100 μL) from each sheep was incubated for 4 h with 0.5 pmol of receptor and assayed by the protein A-Sepharose assay. Receptor alone bound 9.5%, and this background was subtracted from all numbers. Left panel: Sheep which have not been injected (●) with any antigen were compared with sheep which had been injected with nonreceptor antigens (○). Right panel: Four additional sheep were assayed for endogenous antibody (●) and then injected as described under Materials and Methods, either with receptor A (□) or with receptor B (△).

Results

The interaction of sheep sera with chick receptor was first detected by sucrose-gradient centrifugation. Figure 1a,b demonstrates the ability of a crude sheep IgG fraction to increase the sedimentation coefficient of the receptor in gradients containing 0.15 M KCl. All preparations tested demonstrated some ability to interact with the chick receptor at this salt concentration. However, at a higher salt concentration (Figure 1c), only about one-third of the sheep had activity. It is this activity which has been further characterized as described below. These IgG fractions did not react either with free progesterone or with corticosteroid binding globulin (CBG) (Figure 1d,e) and thus do not appear to recognize free steroid or plasma binding proteins. Commercially available rabbit anti-sheep IgG also does not react with chick progesterone receptor (Figure 1f).

It has been shown that *Staphylococcus aureus* protein A specifically binds certain classes of IgG (Forsgren & Sjoquist, 1966; Kronvall & Frommel, 1970) by interaction at the Fab region, thereby leaving the combining site free to react. In order to characterize this receptor binding activity further, crude IgG was incubated with progesterone receptor and assayed by the protein A–Sepharose method. Of the 17 sheep tested, about one-third had an IgG-like molecule which reacted with the chick progesterone receptor as well as with the protein A (Figure 2). The sheep were either positive both by sucrose-gradient analysis in 0.3 M KCl and by protein A–Sepharose or negative in both tests. Since protein A–Sepharose is specific for IgG, it appears that both methods are measuring an IgG which reacts with the receptor. The receptor-complexing activity appears to be an immunoglobulin of the IgG class by two additional tests. First, the activity fractionates with authentic IgG on DEAE-cellulose columns (data not shown). Second, the acid eluates from the protein A–Sepharose columns were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and over 90% of the stained protein was IgG. Neither test above constitutes an absolute

Table I: Interaction of Sheep IgG with Receptors

receptor source[b]	% ^{3}H-labeled receptor bound[a]		
	+IgG	−IgG	difference
chick progesterone	65	12	53
human progesterone	5	5	0
rabbit progesterone	4	3	1
rat estrogen	9	5	4
chick glucocorticoid	12	7	5

[a] Receptor samples (0.1–0.5 pmol) were incubated with 100 μL of crude IgG fraction for 4 h and assayed by the protein A–Sepharose assay. [b] Receptors were prepared as described under Materials and Methods.

proof that the antibody is IgG, since the activity is such a small fraction of the total immunoglobulins. Attempts to use rabbit anti-sheep IgG as a precipitating second antibody were unsuccessful, since enormous amounts of sheep IgG would need to be complexed in order to react a significant fraction of these dilute receptor–sheep antibody complexes.

Earlier work with spontaneous antibodies to estrogen receptors (Fox, 1978) had demonstrated that only animals which had been immunized against other, good antigens contained significant titers of receptor–complexing activity. As a test for this possibility in the present experiments, sera from animals which had never been injected were compared with sera from animals injected with a variety of unrelated antigens, such as human blood antigens. These latter animals were successful producers of specific antibodies of high titer directed against the injected antigens (data not shown). In contrast to the studies of Fox, the data in Figure 2 (left panel) demonstrate that the presence of progesterone receptor binding activity does not correlate with the past immunization history of the animals. In fact, the two animals with the highest titer of progesterone receptor reactivity were not previously immunized.

Figure 2 also demonstrates that immunoreactivity of several animals was not affected by repeated injections with receptors. Two animals which were reactive and two animals which lacked this activity were injected with receptor A or B as described under Materials and Methods. Serum from these animals was assayed after several booster injections over a period of months, but no alteration in the level or type of activity was seen in either pair of animals (Figure 2, right panel). Both female and castrated male animals were used; no correlation with the sex of the animal was found.

Because this IgG activity was not elicited as a result of immunization, a variety of steroid hormone receptors was prepared and tested in order to see if the IgG was recognizing some common feature of all steroid receptors (Table I). Three mammalian progesterone receptors were assayed for cross-reactivity, and none was reactive, suggesting that a common feature of progesterone receptors was not being recognized. Chicken glucocorticoid receptor was tested to see if a common feature of chicken steroid receptor was recognized. Again, the receptor did not react. We do not know whether the chicken estrogen receptor reacts since we do not have a method to obtain high specific activity chicken estrogen receptor. Rat androgen and estrogen receptors were also tested and failed to react. Sucrose-gradient analysis of the estrogen receptor preparation indicated that 4S, 5S, and 8S species were all present and none appeared to react. Thus, the IgG does not seem to be recognizing a common feature of receptors or even of progesterone receptors.

Since all of the analyses were conducted with radioactive ligands to identify complexes, no information was obtained about other antibodies which might react with chicken proteins.

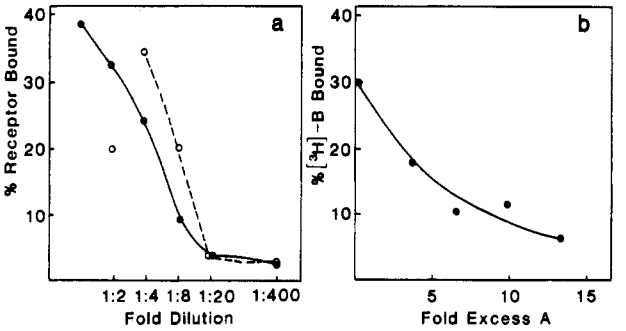

FIGURE 3: Titer of sheep serum for progesterone receptor proteins. (a) The indicated amounts of crude IgG (200 μL of the appropriate dilution) were incubated with 0.2 pmol of A or B and assayed by the protein A–Sepharose column method: (●) A; (○) B. (b) The indicated amounts of unlabeled A were incubated with 0.25 pmol of ^{3}H-labeled B and 150 μL of crude IgG fraction and assayed by the protein A–Sepharose column method.

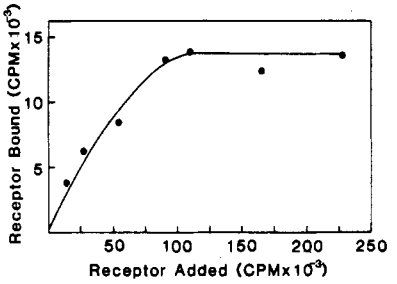

FIGURE 4: Quantitation of receptor binding activity in sheep serum. The indicated amounts of receptor were incubated with 150 μL of crude IgG and assayed by the protein A–Sepharose column method.

Therefore, without further characterization, the antibody can only be used in cases where the receptor is specifically labeled.

Receptor preparations used in the experiments shown in Figure 2 contained a mixture of receptors A and B. In Figure 3, partially purified A and B subunits were tested individually for their reactivity with the crude IgG. Figure 3a shows that both subunits A and B react with the crude IgG fraction and that it has a similar capacity for each of the subunits. Unlabeled A competes with labeled B for serum binding capacity (Figure 3b) as would be expected if the same IgG molecule recognizes both A and B.

In no case was more than 60% of the receptor bound to the IgG. This suggested either that a portion of the receptor molecules was not recognized or that the affinity of the antibody for the receptor was too low to see quantitative binding under the conditions used. Since the antibody was not raised against receptor, the latter seemed likely. Therefore, the K_d for the dissociation of the receptor–antibody complex was measured. First, the number of available binding sites in the IgG preparation was determined by incubating increasing amounts of receptor with a constant quantity of IgG and assaying for bound receptor by the protein A–Sepharose assay. As shown in Figure 4, approximately 13 000 cpm (0.4 pmol) of receptor can be bound by 150 μL of the IgG. Assuming then that 150 μL of IgG contains 0.4 pmol of binding sites, we calculated a dissociation constant for the receptor–IgG complex receptor concentrations below the saturation point. The apparent K_d determined in this manner is 2×10^{-9} M.

Chick cytosol contains a Ca^{2+}-activated protease which cleaves both native receptor subunits into smaller hormone-binding fragments (meroreceptors) and an unknown number of other peptides (Vedeckis et al., 1980; Sherman & Diaz, 1977). ^{3}H-Labeled meroreceptor was prepared as described by Vedeckis et al. (1980) and assayed by sucrose-gradient analysis (data not shown) and by protein A–Sepharose (Figure

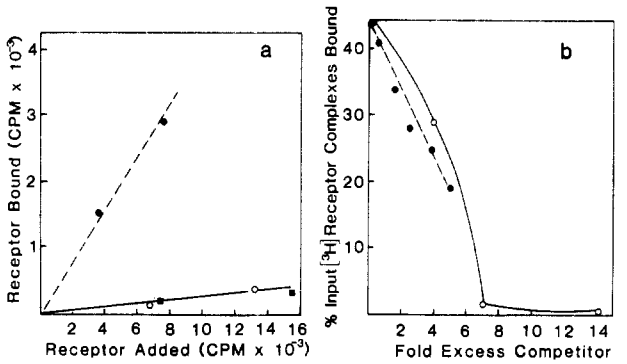

FIGURE 5: Analysis of immunoreactive domains. (a) The indicated amounts of receptor were incubated with 50 μL of crude IgG from sheep R73 and assayed by the protein A–Sepharose method: (●) ^{3}H-labeled receptor + sheep serum; (○) meroreceptor + sheep serum; (■) meroreceptor alone. (b) The indicated amounts of receptor or receptor digest were incubated with 0.2 pmol of labeled receptor and 100 μL of crude IgG and were assayed by the protein A–Sepharose method: (●) + receptor; (○) + receptor digest.

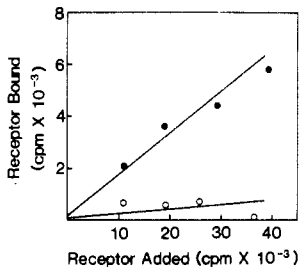

FIGURE 6: Interaction of native cytosol receptor with sheep IgG. The indicated amounts of receptor were incubated with 150 μL of crude IgG in a final volume of 350 μL for 2 h at 4 °C and assayed by the protein A–Sepharose assay. The amount of receptor bound to the protein A–Sepharose in the absence of IgG (10%) has been subtracted from the value for the receptor-bound (●) monomer (○) aggregate.

5a) for its ability to bind to the sheep IgG. In neither case was any binding seen. However, the presence of a non-hormone-binding peptide with antigenic activity was detected by a competition assay (Figure 5b). It was possible that one or more antigenic sites were lost by proteolytic digestion, while one or more were still retained. However, since the digest is as effective as a competitor as is the intact protein, this appears to be unlikely. Therefore, the immunoglobulin appears to interact only with a site or sites on the non-hormone-binding portion of the subunits, distal to the meroprotein portion.

For further characterization of the antibody binding site, the reactivity of the native cytoplasmic AB receptor aggregate was compared with the reactivity of the dissociated proteins. Aggregates were prepared as described under Materials and Methods, and a salt-dissociated solution of the same aggregates was used as the source of control monomers. Figure 6 shows that whereas monomers prepared in this manner react as in the previous experiments, the aggregates show virtually no reactivity. Furthermore, aggregates are unable to compete with monomers for binding in the protein A assay (data not shown). Since these aggregates dissociate with time even in low salt, the small amount of reactivity may be due to small amounts of monomer in the preparation. We conclude that the immunologic domains of B and A are occluded in the native complex.

Discussion

The chick progesterone receptor complexing activity appears both in nonimmunized animals and in animals immunized against other antigens. About one-third of the sheep tested had this activity, and no correlation with age or with sex of the animal was found. Neither injection of receptors into sheep with endogenous complexing activity nor injection of sheep lacking this activity resulted in an increase in titer or in the appearance of new antibodies. The serum from three animals containing this activity was examined, and no differences in the specificity of any type were seen. Thus, it is probable that all reactive sheep contain the same activity. It is possible that sheep are exposed to a protein with some similarity to the chick progesterone receptor and therefore already contain an immunoglobulin which reacts with the receptor and prevents the sheep from responding to injection. Fox (1978) has reported a serum component, designated 5S-CA, found only in immunized animals which forms a complex with the 5S form of estrogen receptors and shifts its position in sucrose gradients. The 5S-CA activity appears in response to a variety of unrelated antigens and reacts with the 5S form of estrogen receptor from all animals tested. We did not detect 5S estrogen receptor binding activity in serum containing progesterone receptor binding activity. The progesterone receptor binding activity is very specific for the chick progesterone receptor and did not interact with the other receptors tested. Moreover, it was found in animals which were not specifically immunized with any antigen.

Rabbit anti-sheep IgG, which is similar to described sources of 5S-CA (but may not contain 5S-CA), does not react with progesterone receptor (Figure 1f), when used in amounts which should show 5S-CA activity (Fox, 1978). All of these results suggest that the progesterone receptor complexing activity is not identical with the 5S-CA activity reported by Fox. It should be emphasized that the titers of these activities are very low and would probably not be observed in the presence of a good antibody response. The apparent dissociation constant for the complex, $K_d = 2 \times 10^{-9}$ M, is of the same order of magnitude as the concentration of receptor and antibody sites in the incubations and therefore is consistent with the incomplete complexing of the receptor in the assay.

The endogenous complexing activity proved to be useful as a tool to study the structure of the receptor. A and B react equally with the IgG. Furthermore, the A protein competes with B for the IgG, indicating that at least portions of these two proteins are quite similar. We had shown previously that the Ca^{2+}-activated protease cleaves the two subunits into similar if not identical hormone binding fragments (Vedeckis et al., 1980), but little was known about the other portions of the proteins. Recent studies have shown that the peptide maps of ^{125}I-labeled tryptic digests of the two proteins are quite different (N. L. Weigel, unpublished experiments). However, characterization of the reaction of the Ca^{2+}-activated protease digest with the antibody demonstrates first that the remaining portions of the protein are not completely degraded and second that there are also similarities between the non-hormone-binding portions of the A and B subunits.

Finally, the lack of reactivity of the intact receptor AB complex suggests that the antigenic recognition site or sites are occluded in the associated form of the receptor.

Recently, Logeat et al. (1981) have reported production of a rabbit progesterone receptor antibody in goats. This antibody is also of rather low titer, although not as low as the spontaneous antibody to chicken receptor. It is of interest that the chicken progesterone receptor did not cross-react with the rabbit receptor antibody while mammalian progesterone receptors did cross-react. We do not know if the endogenous chick progesterone antibody interferes with production of a specifically induced antibody to the receptor or if some other characteristic of the receptor makes it so nonantigenic.

Although the antibody is of low titer, serum containing the activity is readily available. Therefore, we plan to purify the antibody by affinity chromatography on a receptor B column as has been done for a low titer, low affinity calmodulin antibody (Dedman et al., 1978). This will permit us to perform experiments with unlabeled receptor and to isolate the portion of the receptor containing the antigenic site.

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Acyl Chain Order and Lateral Domain Formation in Mixed Phosphatidylcholine–Sphingomyelin Multilamellar and Unilamellar Vesicles†

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ABSTRACT: The phase behavior of mixtures of dimyristoylphosphatidylcholine (DMPC) with *N*-palmitoylsphingosinephosphorylcholine (C_{16}SPH) has been investigated in both small unilamellar and large multilamellar vesicles. The steady-state fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) has been used to detect temperature-induced structural changes in these membranes. In addition, electron microscopy has revealed vastly different fracture-face morphologies for large multilamellar vesicles "jet-frozen" from different temperatures. These data have been interpreted in terms of proposed phase diagrams for this lipid mixture. The shapes of the proposed phase diagrams have led us to conclude that phosphatidylcholine and sphingomyelin species of similar acyl chain length mix freely in both highly curved and uncurved bilayers, except at temperatures at which both lipids are in low-temperature, ordered phases. In addition, the similarity of these phase diagrams to phase diagrams for analogous mixtures of pure phosphatidylcholines suggested that sphingomyelin and phosphatidylcholine species might substitute for each other in supporting the lamellar phase necessary to cell membrane structure. Finally, the anisotropy of DPH fluorescence was found to be essentially invariant with sphingomyelin content at temperatures just above and below the solid–liquid phase separation in small unilamellar vesicles. This demonstrates that the sphingomyelin backbone, per se, does not order the membrane bilayer. These results are discussed in terms of the possible role of sphingomyelin in controlling acyl chain order within mammalian cell membranes.

The phase behavior of many of the component phospholipids of mammalian membranes has been studied extensively in recent years with a view toward defining the role of different phospholipid species in establishing membrane structure. While the ability of some species to form nonlamellar phases may be important to specialized membrane functions (Cullis & DeKruijff, 1979), those species that spontaneously form lamellar mesomorphic phases in water should stabilize the essential bilayer structure of cell membranes. The choline phosphatides phosphatidylcholine and sphingomyelin are major components of mammalian membranes that form lamellar phases in excess water. Changes in the ratio of these two lipids have been correlated with the occurrence of various tissue malfunctions (Barenholz & Thompson, 1980) such as aging (Rouser & Solomon, 1969), atherosclerosis (Small & Shipley,

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