SPINIMMUNOASSAY OF PROGESTERONE

Robert Wei and Ramona Almirez

Research Division, Electronucleonics, Inc.
Silver Spring, Maryland

Received November 7, 1974

Summary

A spin-labeled derivative of progesterone was prepared: 3(progesterone -11∝-hemisuccinyl)-3 methylamino-2,2,5,5, tetramethyl pyrrolidine-1-oxyl. A corresponding antibody was produced by inoculating rabbits with progesterone-11∝-hemisuccinyl-(bovine serum albumin). These materials were then used in developing a method to measure progesterone. The method was shown to be specific for progesterone and capable of measuring concentrations as low as 0.1 µg/ml.

Introduction

Since the report on the immunoassay of insulin by Berson and Yalow(1), Radioimmunoassay(RIA) methods have been extensively used in clinical laboratories for determining hormones and other components in body fluids. These methods are usually specific and highly sensitive. However, RIA methods generally require rather long incubation periods and the separation of bound antigen from unbound antigen prior to the measurement of radioactivity. By contrast, spinimmunoassay(SIA) is almost instantaneous and does not require a comparable manipulation. Furthermore, unlike RIA, SIA presents no safety hazard. In spite of these advantages, SIA methods have thus far been limited to determining opiates(2). Recently, Chignell et al(3) reported a spin-labeled method measuring sulfonamide drugs at levels ranging from 0.3 µg/ml.

The purpose of this communication is to describe briefly the development of a SIA method for progesterone. References (2) and (4) are recommended for general background and applications related to spin-label materials.

Materials and Methods

Progesterone-11α-hemisuccinate(Steraloids, Inc., Pawling, N.Y.) was coupled to bovine serum albumin by the mixed anhydride method(5). Material of low molecular weight was removed by dialysis; the residue was emulsified with Freund's adjuvant and injected into rabbits. Blood was drawn from the rabbits about 2 months later and the serum containing the antibodies was separated.

A spin-label, 2,2,5,5, tetramethyl-3-amido-pyrrolidine-1-oxyl (Aldrich Chemical Co., Milwaukee, Wi.), was reduced to 2,2,5,5, tetramethyl 3-methylamino-pyrrolidine-1-oxyl with lithium aluminum hydride(6). The latter was condensed with progesterone-11α-hemisuccinate using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline as a coupling agent to produce the spin-labeled derivative of progesterone.

Results

Electron spin resonance(ESR) spectra were obtained with a modified E-4 spectrometer(Varian). The diluent used in preparing solutions for examination contained, per liter, 0.14 mole sodium chloride, 10 mmole sodium phosphate buffer(pH 7.3), and 1 g of sodium azide as preservative.

Figure 1-A shows the spectrum obtained from labeled progesterone, with three sharp peaks(i, ii, iii), as expected from a rapidly tumbling spin-label. When the labeled progesterone molecules are bound to antibody, the tumbling of these complexes is slower and the peaks in the spectrum(fig. 1-B) are lower and broader. If unlabeled progesterone is now added, it displaces some of the labeled progesterone from the antibodies and the high peaks reappear almost instantaneously(fig.1-C). A sharpness of the peaks(i, ii, iii) in figure 1-B is attributable to the presence of a small amount of unbound labeled progesterone. This provides the basis for measurement of progesterone concentration. For analytical purposes, only the iii peak is measured(fig.D-F).

Two experiments were carried out to test the specificity of the new analytical method for progesterone. In the first experiment, the decrease in peak height was determined when 10 μl of a non-corresponding antiserum was mixed with 25 μl of 5.5×10^{-7}M spin-labeled progesterone and equilibrated for 2 minutes. The results obtained with three such antisera are listed in Table I. They show that a non-corresp-

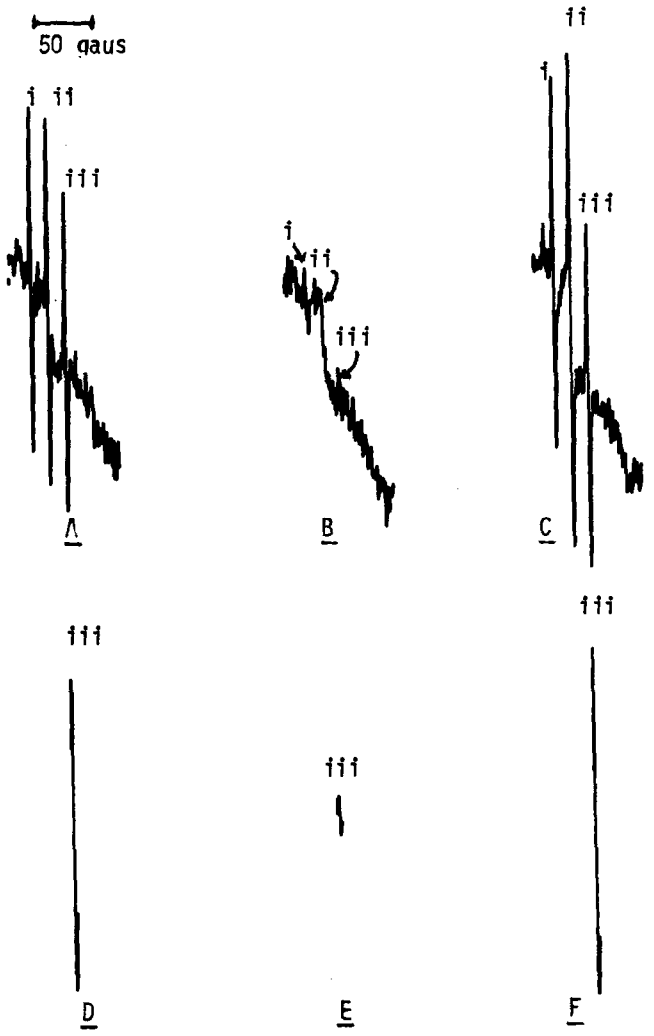

Figure 1. ESR spectrum of Spin-labeled Progesterone. A, spectrum of spin-labeled progesterone in regular diluent. B, a composite spectrum of antibody-bound spin label and unbound spin-label. C, addition of progesterone(2 μg/ml) to antibody/spin-label mixture. Spectra D-F corresponds to A-C at "normal" position on E-4 spectrometer.

---

onding antiserum may bind about 10% as much labeled progesterone as does the corresponding antiserum. Perhaps half of the 10% can be attributed to binding by the albumin in the antiserum, rather than antibodies per se. This is indicated by the last entry in the table, the antiserum for which was first treated with ammonium sulfate to remove the albumin before mixing with the solution of spin-labeled progesterone.

TABLE I. Binding of Spin-labeled Progesterone by Corresponding and Non-corresponding Antisera

Antiserum Corresponding to ;	Peak Height (Arbitrary Units)	Relative Binding, %
Nothing (Diluent)	13.0	0.0
Progesterone-11-α-Hemisuccinate	0.5	100.0
3-O-Carboxymethyl-17β-estradiol	11.9	8.4
3-O-Carboxymethyl morphine	11.7	10.0
Testosterone-3-oxime	11.7	10.0
Testosterone-3-oxime (Albumin removed)	12.3	5.2

In the second experiment, 20 μl of a standard solution of a steroid was mixed with 10 μl of the progesterone antiserum. Then 10 μl of a spin-labeled progesterone solution was added and the peak height was determined. The results obtained with unlabeled progesterone and with five extraneous steroids are listed in Table II and show that interference from the extraneous steroids is negligible.

The sensitivity of the new method is illustrated in figure 2, which is a calibration curve developed with standard solutions of progesterone in our regular diluent. A similar curve was obtained when standard solutions were made up in pooled male serum. Although not shown in the figure, we found it possible, by using more dilute antibody, to detect concentrations of progesterone as low as 20 to 50 nanograms/ml.

TABLE II. Cross Reactivities of Progesterone Antiserum With Other Steroids

Steroids	Concn. of Soln., ug/ml	Peak Height (Arbitrary unit)	Cross Reactivity,%
Progesterone	0.0	3.5	-
	0.5	6.0	-
	1.0	8.3	-
Deoxycortico-sterone	1.0	3.6	3
	5.0	4.5	
	10.0	5.3	
Pregnane-diol	1.0	3.8	3
	5.0	4.8	
	10.0	5.4	
Cortisol	1.0	3.5	<1
	5.0	3.6	
	10.0	3.5	
Estradiol	1.0	3.3	<1
	5.0	3.5	
	10.0	3.5	
Testoster-one	1.0	3.5	<1
	5.0	3.7	
	10.0	3.5	

Discussion

At present, RIA is probably the method of choice for measuring biological components at concentrations of nanogram/ml and below. However, at concentrations near 0.1 μg/ml, SIA is better suited for routine use because of its speed and simplicity. The progesterone method described here, because of its limitation in sensitivity, will probably be useful only in certain situation(e.g., adrenal hyperplasia, a certain phase of the menstral cycle, etc.). However, the SIA principle appears applicable to a host of analytical problems, some of which are now being explored in this laboratory.

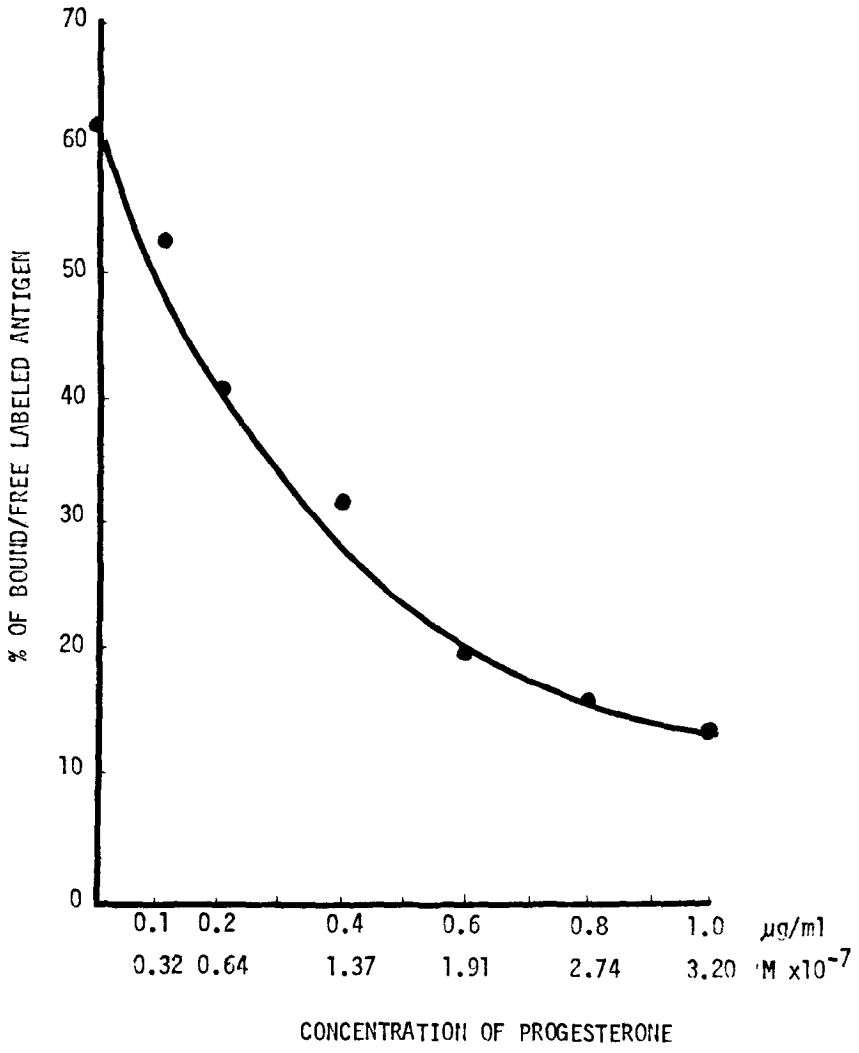

Figure 2. Standard Curve for the Assay of Progesterone. 20 µl of progesterone calibrators, prepared in regular diluent, were mixed with 10 µl of $(NH_4)_2SO_4$-treated antiserum. 20 µl of the mixture was added to 10 µl of spin-labeled progesterone(1.1×10^{-6}M)

References

1. Berson, S.A., and Yalow, R. S., Immunoassay of Plasma Insulin. In Immunoassay of Hormones, Ciba Found. Colloq. Endocrinol., 14, 182(1962)

2. Leute, R. K., Ullman, E. F., Goldstein, A., Nature(New Biology), 236, No.64, 93(1972)

3. Chignell, C. F., and Starkweather, D., Biochim. Biophys. Acta, 271, 6(1972)

4. Swartz, H. M., Bolton, J. R., and Borg, D. C., Biological Applications of Electron Spin Resonance, Wiley-Interscience, New York-London(1972)

5. Williams, C. A., and Chase, M. W., Methods in Immunology and Immunochemistry, Vol. 1, 147, Academic Press, New York and London(1967)

6. Hsia, J. and Piette, L., Arch. Biochim. Biophys., 129, 296(1969).