PROGESTERONE IN THE UTERUS AND THE PLASMA
II. THE ROLE OF HORMONE AVAILABILITY AND METABOLISM ON SELECTIVE BINDING TO UTERUS PROTEIN

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SUMMARY : In the rat uterus cytosol, there is a progesterone and corticosteroid binding protein identical or very similar to plasma corticosteroid binding globulin (CBG) (1,2). When the uterine horns were incubated for 30 min. at 37° with the corresponding steroid, progesterone, but not cortisol and little corticosterone were bound to the CBG-like protein. Analysis of tissular radioactivity and steroid metabolism showed that steroid incorporation (greater for progesterone) and steroid catabolism (faster for cortisol) were responsible of these binding differences. Experiments also gave evidence for the intracellular localization of the uterine CBG-like protein. The same process may also operate in vivo to select the bound hormones, since after injection of the radioactive hormones, the uterine CBG-like protein was labelled by progesterone, relatively less by corticosterone and not by cortisol.

A progesterone and corticosteroid binding protein identical or very similar to plasma corticosteroid binding globulin (CBG) has been found in the rat uterus cytosol (1,2), whereas it was absent or in very low concentration in non-target organs. The concentration of this CBG-like protein in the uterus was under the influence of the rat endocrine status and was especially dependent on estrogen priming. Its presence in the uterus could not be simply ascribed to blood or plasma contamination. All these previously reported studies had however been done by incubating directly the uterine cytosol with ^{3}H-steroids. In this condition, some important physiological features, as permeability and metabolism, are bypassed. New experiments, reported here, using organ incubation and in vivo injection of ^{3}H-steroids allowed an analysis of the role of these factors in binding and gave supplementary arguments to show that the binding protein is probably intracellular.

I - Incubation of uterine horns with radioactive hormones.

All experiments included an internal control, since from the uterus of each animal one horn was used for incubating with ^{3}H-progesterone and the opposite horn for incubating with ^{3}H-corticosteroid. Incubations were performed at 37° under agitation in a Dubnoff incubator in Krebs-Ringer

phosphate glucose (1 mg/ml), for 30 min. (after this time the rate of incorporation of radioactive progesterone or corticosteroids became very slow). Steroid concentration was 10 nM and there were 5 uterine horns from 250 g Wistar rats (at random period of the cycle) in each 5 ml incubation. The cytosol obtained after homogenization in Tris HCl 10 mM, $CaCl_2$ 3 mM, pH 7.4 buffer (1 ml/uterine horn) and centrifugation at 105,000x90 gxmin was tested for radioactivity binding by Sephadex G-25 chromatography and sucrose gradient centrifugation.

After incubation with ^{3}H-progesterone, macromolecule-bound radioactivity was observed whereas it was not after ^{3}H-cortisol incubation (Fig. 1 upper part). In the case of ^{3}H-corticosterone, it was 30-50 % of what was observed with ^{3}H-progesterone. In sucrose gradient experiments, the peak of radioactivity was slightly lighter than bovine serum albumin (BSA), corresponding to the previously described CBG-like protein.

This binding difference between progesterone and corticosteroids observed after organ incubation was in contrast with the binding of these steroids after the direct incubation of the uterine cytosol (Fig. 1, lower part). In the latter case, cortisol (and corticosterone) was even more bound than progesterone.

This discrepancy was explained by the analysis of steroid distribution and metabolism under the incubation conditions. As reported in Table 1, total radioactivity in the uteri was markedly higher after incubation with ^{3}H-progesterone than after incubation with ^{3}H-corticosteroids. From both the incubation medium and the cytosol, steroids were extracted, chromatographed and analyzed by radioactivity scanning. For progesterone, a silicagel thin layer system with benzene 3-ethylacetate 2 was used, and for corticosteroids, a paper chromatography system with chloroform formamide. The regions of progesterone and cortisol, respectively, were eluted and these steroids identified by cristallisation until constant specific activity after dilution with the correspondant authentic steroid. Results reported in Table II indicated that only a small part of the incubated ^{3}H-progesterone was metabolized and that the metabolite percentage was identical in the incubation medium and in the cytosol. With cortisol, the metabolism was more extensive and if unchanged cortisol represented half of the radioactivity in the incubation medium, it was only 10 % in the cytosol. It was calculated that 25 % of the ^{3}H- progesterone initially incubated with the uteri was finally found as such in the cytosol, whereas in parallel experiments only 0.9 % of the ^{3}H-cortisol was found as unchanged cortisol in the cytosol. Experiments with ^{3}H-corticosterone gave intermediary results. The differences in radioactivity content (smaller for cortisol)

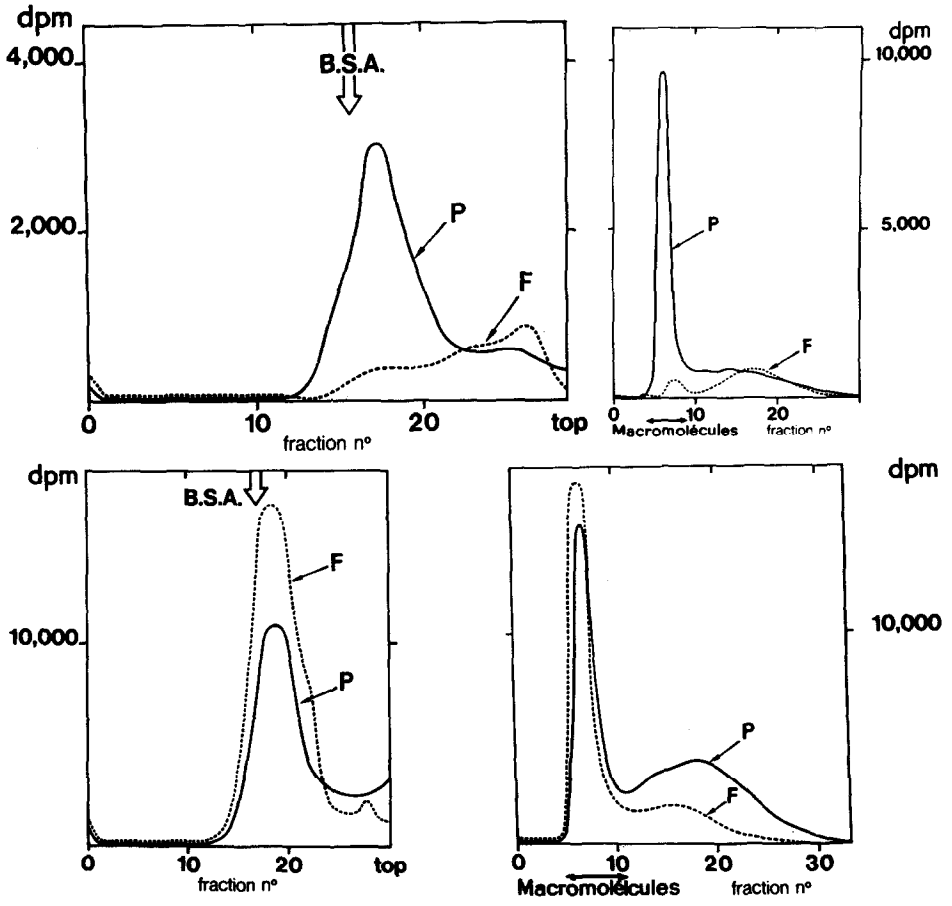

Figure 1

^{3}H-PROGESTERONE (P) AND ^{3}H-CORTISOL (F) BINDING TO RAT UTERUS CYTOSOL

Upper left part : SUCROSE GRADIENT AFTER INCUBATION OF UTERI. 0.2 ml of the cytosol was layered on top of a 5-20 % sucrose gradient. Centrifugation 18 h. at 0° and 50,000 rpm in a SW 65 rotor.

Upper right part : SEPHADEX G-25 AFTER INCUBATION OF UTERI. 0.2 ml of the cytosol was chromatographed through a Sephadex G-25 coarse microcolumn (height : 12 cm, diameter : 0.6 cm, 0.21 ml fractions, flow : 0.14 ml/min.) at 4°.

Lower left part : SUCROSE GRADIENT AFTER DIRECT INCUBATION OF CYTOSOL. 5 horns from 250 g Wistar rats were rinsed in cold buffer and homogenized in 5 ml of Tris HCl 10 mM, $CaCl_2$ 3mM, pH 7.4. The cytosol was incubated with 10 nM ^{3}H-steroid (SA = 20 Ci/mmole), 30 min. at 25° with agitation and 60 min. at 4°. 0.2 ml was layered on top of a 5-20 % sucrose gradient. Centrifugation 18 h. at 0° and 45,000 rpm in a SW 65 rotor.

Lower right part : SEPHADEX G-25 AFTER DIRECT INCUBATION OF CYTOSOL : Incubation and chromatography as in lower left and upper right, respectively.

TABLE 1

DISTRIBUTION OF RADIOACTIVITY (± S.E.M.) AFTER INCUBATION OF RAT UTERI WITH ^{3}H-PROGESTERONE AND ^{3}H-CORTICOSTEROIDS.

	(N^x)	Incubation medium	Cytosol	105,000 g pellet
Progesterone	4	44.2 ± 1.6	34.8 ± 2.1	20.5 ± 1.3
Cortisol	4	89.7 ± 0.4	8.8 ± 0.2	1.6 ± 0.2
Corticosterone	1	74	23.4	2.6

N^x : Number of experiments (each with a pool of 5 uterine horns)

Table 2

METABOLITES OF CORTISOL AND PROGESTERONE

(Percent of radioactivity in incubation medium and cytosol)

	progesterone incubation			cortisol incubation				
	more polar	PROGESTERONE	less polar	more polar	CORTISOL	(x)	cortisone	less polar
Incubation medium	4	68	28	4	51	0	24	21
Cytosol	5	72	23	32	10	0	25	33

(x) intermediary zone between cortisol and cortisone

and steroid metabolism (faster for cortisol) probably explain why after organ incubation cortisol was not bound to the CBG-like protein whereas progesterone was. When the cytosol was directly incubated with the steroid, there was nearly no metabolism and of course the destruction of cellular organization as obtained in cytosol incubation suppressed any difference due to transport characteristics (possibly on the way in or out of the hormone or of its metabolites).

Another set of experiments were performed with castrated animals

whose uteri contained less CBG-like protein (1,2). The binding of progesterone was smaller, but the binding of cortisol greater than in normal animals and a slower catabolism of cortisol was observed. Estrogen treatment nearly completely restored the situation existing in normal animals.

The present results strongly suggested the intracellular localization of the uterine CBG-like protein. If it was directly accessible to the steroids present in the incubation medium, there should have been a greater binding of cortisol than of progesterone since 46 % of the ^{3}H-cortisol and only 30 % of the ^{3}H-progesterone initially incubated were present as unchanged steroid in the incubation medium at the end of the experiment. It was not the case, and reciprocally, the amount of progesterone and cortisol bound to the CBG-like protein was related to their respective concentrations in the cytosol (25 % and 0.9 % of the initially incubated steroid as calculated above). These results indicated that there was a permeability barrier between the incubation medium and the CBG-like protein. To further test this possibility, the cytosol obtained after an organ incubation with ^{3}H-

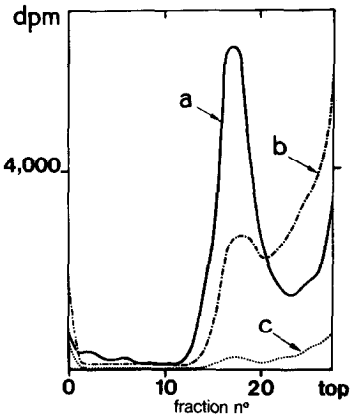

Figure 2

ACCESS OF ^{3}H-CORTISOL TO THE CBG-LIKE PROTEIN IN THE RAT UTERUS

5 uterine horns were incubated in Krebs-Ringer phosphate glucose ^{3}H-cortisol 10 nM. The cytosol was obtained and the incubation medium saved. Then incubations were performed for 60 min. at 0° with mild agitation in a Dubnoff incubator:

a : 1 ml cytosol + 1 ml incubation medium
b : 1 ml incubation medium + 1 ml homogenization buffer
c : 1 ml cytosol + 1 ml Krebs-Ringer phosphate glucose

0.2 ml of each incubation was then centrifuged through a 5-20 % sucrose gradient (19 h. 30, SW 65 rotor, 45,000 rpm at 0°).

cortisol (see Table 1) was reincubated with the incubation medium. Sucrose gradient analysis indicated practically no binding in the original cytosol of the incubated uteri (Fig. 2c), only a small CBG-like peak in the incubation medium (Fig. 2b) (due to the leaking of a small amount of the protein out of the tissue), and a very large radioactive peak after reincubation of the cytosol with the initial incubation medium (Fig. 2a). This experiment showed that the permeability barrier between the incubation medium and the CBG-like protein was abolished by the homogenization of the tissue.

II. In vivo experiments

50 µcuries of ^{3}H-progesterone (SA = 33,3 Ci/mmole) were injected intravenously into two 250 g Wistar rats castrated for 7 days and primed with 0.2 µg estradiol per day for 3 days before the experiment. After 10 min., the uterus, one kidney, thigh muscle, small intestine and some blood were obtained. The cytosol of different organs and the plasma were prepared and diluted with Tris HCl 10 mM $CaCl_2$ 3 mM pH 7.4 buffer to obtain approximatively the same protein concentration (4-8 mg/ml) and analyzed by sucrose gradient centrifugation (Fig. 3, left side). The results were normalized per mg of protein. A 4 S radioactive peak was observed in the uterus cytosol, a smaller and identical peak in the plasma but no bound radioactivity was detected in the other organs cytosol. After injection of an identical dose of ^{3}H-cortisol (SA = 33,3 Ci/mmole), no bound cortisol was observed in the uterus whereas in the plasma an important radioactive peak could be observed (Fig. 3, right side). With ^{3}H-corticosterone, a small binding in the uterus and an important binding in the plasma were observed. Therefore, these in vivo experiments confirmed the organ incubation experiments showing again a preferential binding of progesterone when compared to corticosteroids by the CBG-like uterus protein.

The origin of this binding protein is unknown. It may be synthetized in the uterine cells or be of plasmatic origin, perhaps imported because of the special permeability changes due to the estrogens (3,4). In any case, its localization is tissue specific since as previously shown (1,2), it is present in the uterus in high concentration, whereas it is absent (or in very small concentration) in the kidney or diaphragm. These results are confirmed by the above reported in vivo experiments.

The physiological role of this binding protein in the uterus cannot be defined at the present time. It cannot be said if it has a role in the passage of progesterone from the blood into the uterus, in its intracellular transport, and if it plays a role in any further event implicated in this steroid action.

This progesterone binding in the rat uterus differs from that found for estradiol, androstanolone (metabolite of testosterone) and aldosterone in

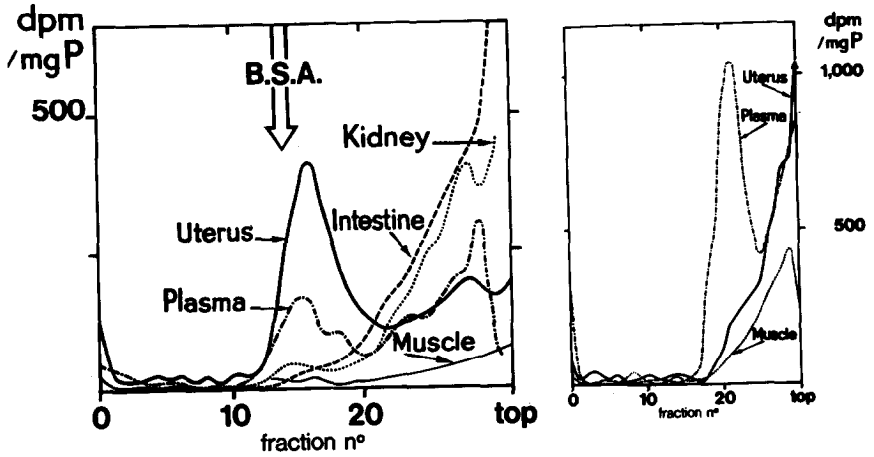

Figure 3

SUCROSE GRADIENT PATTERN OF RADIOACTIVITY BINDING IN PLASMA AND VARIOUS ORGAN CYTOSOL AFTER IN VIVO INJECTION OF ^{3}H-STEROIDS TO RATS.

Left side = PROGESTERONE. 0.2 ml of diluted plasma or cytosol were ultracentrifuged 18 h. at 48,000 rpm with a SW 50-1 rotor (0°) in a 5-20 % sucrose gradient. Protein concentration was measured with the Folin-Lowry technique and data are dpm/mg of protein.

Right side = CORTISOL. Ultracentrifugation at 45,000 rpm, 18 h. in a SW 65 rotor.

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their respective target tissues, where a specific binding protein different from the corresponding plasma binding protein was observed. However, it cannot be excluded that in the uterus, another different progesterone or progesterone metabolite binding protein coexists with the CBG-like protein. The presence of the latter in the uterus reintroduces the discussion of the role of the hormone plasma binding proteins, which could have a more "positive" significance than to be simply, as usually admitted, a circulating reservoir. This work indicates also that the binding characteristics of a protein, as determined in acellular experiments, have to be reconsidered at the tissular and in vivo level before any interpretation is proposed.

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