HORMONE-DEPENDENT SYNTHESIS AND SECRETION OF UTEROGLOBIN IN ISOLATED RABBIT UTERUS

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1. Introduction

During early pregnancy the protein pattern in the uterine secretion of the rabbit is dominated by the presence of a small protein called uteroglobin [1] or blastokinin [2], which binds progesterone with rather high affinity [3–5], and seems to influence blastocyst development and implantation [2,6,7]. Although uteroglobin has been detected in small amounts in the uterus of normal oestrus and estradiol treated rabbits [8,9], progesterone is required to obtain the pattern of uteroglobin secretion characteristic of the early pregnancy [1,10]. There are reports in the literature that uteroglobin is present in the endometrial cells [8], and that the uterus of pregnant rabbits can incorporate radioactive amino acids into uteroglobin in vivo [11]. We were interested to know whether isolated uteri can synthesize and secrete uteroglobin in vitro, and how the ovarian hormones influence this process. In this paper we present evidence showing that uteroglobin can be synthesized and secreted in isolated uteri, and that there is a correlation between the ability of the endometrium to produce uteroglobin in vitro, and the amount of this protein present in the uterine secretion in vivo.

2. Materials and methods

Female rabbits of various breeds with an average weight of 3 kg were daily injected intramuscularly with estradiol-17β (100 μg) or progesterone (5 mg) in sesame oil as indicated in the text. 24 h after the last injection the animals were killed and the uterine luminal fluid was collected by flushing the uteri with 5 ml per horn of 20 mM Tris–HCl, pH 7.5, containing 0.15 M NaCl [5]. After dialysis against deionized water and subsequent lyophilization the protein pattern was analyzed by polyacrylamide gel electrophoresis [12]. Protein determination was performed according to Lowry et al. [13].

The perfusion of the uterine lumen was performed in a recirculating system maintained at 37°C with the uteri immersed in 20 ml of Hank's balance salt solution. Oxygenation took place in a dropping device where a mixture of 95% O_2 and 5% CO_2 was blown. The system was filled under sterile conditions with 6 ml Eagle's essential spiner medium deprived of leucine and supplemented with $NaHCO_3$ (1.2 mg/ml), penicillin (100 μg/ml), streptomycin (100 μg/ml), and mycostatin (20 μg/ml). The recirculation rate was 20 ml/h. After 5 min. progesterone was added to a final concentration of 10^{-5} M, and 5 min later 50 μCi of L-[4,5-^{3}H]leucine, specific activity 54 Ci/mmol. After recirculating for 3 h the medium was collected, clarified by centrifugation (5000 g/min), and passed through a column of Biol-Gel P2 (100–200 mesh, 2.0 x 50 cm) equilibrated with 0.1 M $(NH_4)HCO_3$. The radioactive protein peak was pooled, lyophilized, and resuspended in 1 ml of 0.1 M $(NH_4)HCO_3$. The protein pattern was analyzed by chromatography on a column of Sephadex G-75 fine (1.5 X 150 cm) equilibrated with 0.1 M $(NH_4)HCO_3$, which had been standardized with [^{3}H]leucine and purified uteroglobin [5]. The radioactivity was determined in 0.5 ml of each fraction.

Identification of uteroglobin in the eluate was performed by polyacrylamide gel electrophoresis [12,14]. The radioactivity was determined by incubating two slices (1 mm each) in 0.5 ml of 30% H_2O_2 at

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70°C for 7 h, and adding 5 ml of liquid scintillation mixture (5 g PPO, 500 mg POPO, 1 liter toluol, 500 ml Triton X-100). Before slicing the gels were stained and scanned at 600 nm.

Additional identification was achieved by immunoprecipitation with monospecific antibodies against purified uteroglobin prepared in guinea pigs [5]. Aliquots of the different fractions were incubated with 20 μl antiserum, first at room temp. for 60 min and then at 4°C for 48 h. Sufficient gamma globulin obtained from rabbits immunized with purified guinea pig gamma globulin was added and incubation continued for 24 h at 4°C. The mixture was centrifuged at 15 000 *g*/10 min through 1 vol of 0.5 M sucrose in 20 mM sodium phosphate buffer pH 7.6, containing 0.15 M NaCl, 0.5% Triton X-100 and 0.5% sodium deoxycholate. The pellets were washed in 20 mM phosphate buffer, pH 7.6, containing 0.15 M NaCl, and used for radioactivity measurements or gel electrophoresis [14].

3. Results

The densitometric tracings of the gel electropherograms of the proteins in the uterine fluid of the three groups of animals studied are shown in fig.1. No uteroglobin peak is observed in the control uterine fluid, whereas in animals treated with 2 daily injections of estradiol 2–3% of the Coomasie blue staining was localized in the uteroglobin area. In the third group, treated with 2 daily injections of estradiol followed by 4 daily injections of progesterone the uteroglobin peak accounted for 42–49% of total protein staining.

In these three experimental groups, the incorporation of [^{3}H]leucine into acid insoluble material of the perfusion medium was linear up to 4 h of incubation, and was over 90% inhibited by puromycin (40 μg/ml). Fractionation of the radioactive proteins in columns of Sephadex G-75 shows that no detectable uteroglobin is synthesized in the control group, whereas 2–3% of the radioactivity incorporated into secreted proteins by the uteri of estradiol-treated rabbits elutes as uteroglobin (fig.2a,b). In the group treated with estradiol and progesterone the radioactive peak in the uteroglobin position was the most prominent in the elution profile, accounting for 45% of the total protein radioactivity (fig.2c). In absolute terms, the amount of

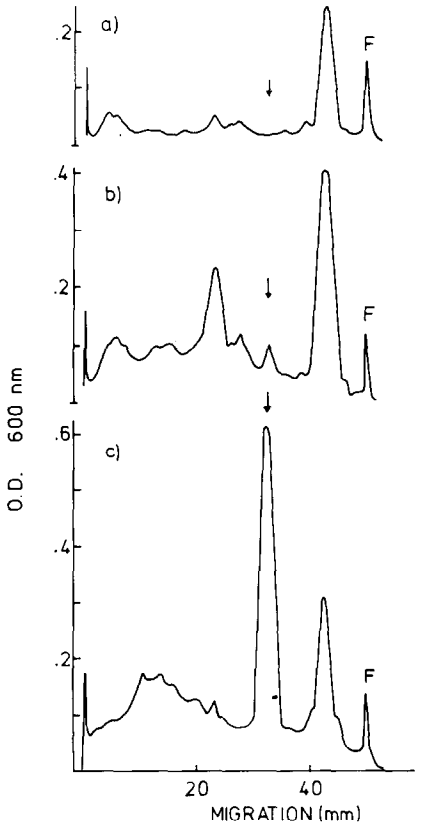

Fig.1. Gel electrophoresis of the proteins in the uterine secretion. The lyophilized uterine luminal fluid was resuspended in 200 μl distilled water, and aliquots were used for electrophoresis in 7.5% polyacrylamide gels at 8.9 [12]. After staining and destaining the gels were scanned at 600 nm. The arrows indicate the position of uteroglobin, and the dye front is labeled 'F'. (a) Control animals; (b) Animals treated twice with estradiol; (c) Animals treated twice with estradiol and 4 days with progesterone.

uteroglobin synthesized and secreted per uterus augmented 30-fold after progesterone treatment. A very similar picture was obtained when the perfusion medium was submitted to immunoprecipitation with monospecific antibodies against uteroglobin (table1).

In addition to the immunoprecipitation, further identification of newly synthesized uteroglobin was achieved by polyacrylamide gel electrophoresis of the material eluting from Sephadex in the position of uteroglobin. Under non-denaturing conditions most of the radioactivity in this fraction coelectrophoresed

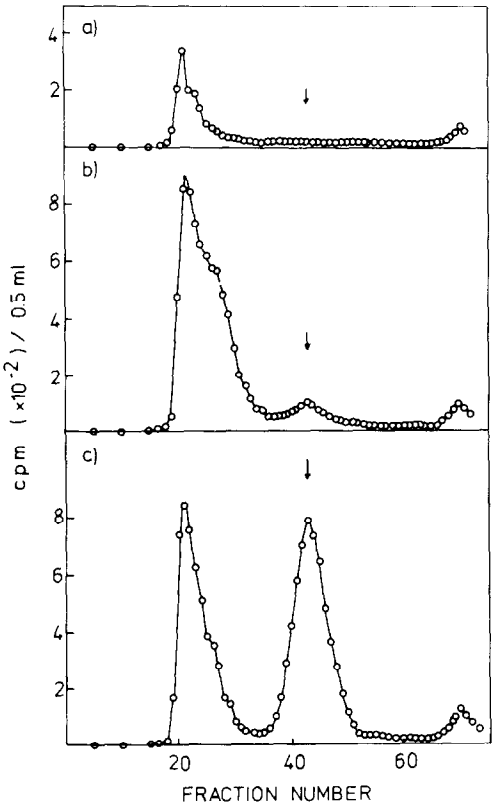

Fig.2. Chromatography on Sephadex G-75 of the proteins synthesized and secreted in vitro. The radioactive proteins in the perfusate were chromatographed in a column of Sephadex G-75 (see Methods). The figure shows the radioactivity in the eluate. The elution position of uteroglobin is indicated by arrows. The small peak around fraction 70, represents free [^{3}H]leucine. (a) Control animals; (b) Animals treated twice with estradiol; (c) Animals treated twice with estradiol and 4 days with progesterone.

with authentic uteroglobin (fig.3a). In the presence of 1% SDS [14], uteroglobin migrates as a single band in the mol. wt region of 12 000. After treatment with 2% 2-mercaptoethanol at 90°C for 5 min it migrates faster (mol. wt/below 8000), probably because it dissociates into two smaller components of similar mol. wt [5]. Fig.3b and c show that in both cases, the radioactivity migrates with the uteroglobin marker.

The data presented above demonstrate that the uteroglobin found in the uteri of estrogen treated animals originates in the endometrium and is not exclusively derived from the oviduct, where estrogen induces the synthesis of a very similar protein [15].

After progesterone treatment of estrogenized rabbits uteroglobin becomes the dominant protein in the uterine secretion, and is also the main product synthesized and secreted by isolated uteri. In conjunction with the puromycin sensitivity, these data demonstrate that progesterone stimulates the synthesis and secretion of uteroglobin. Measurements of the amount of newly synthesized uteroglobin in the cytosol of the endometrium have failed to show an accumulation of the protein, supporting the idea that secretion takes place soon after synthesis is completed. This assumption is in agreement with the close correlation between the uteroglobin content in the uterine secretion and the amount of uteroglobin synthesized in vitro.

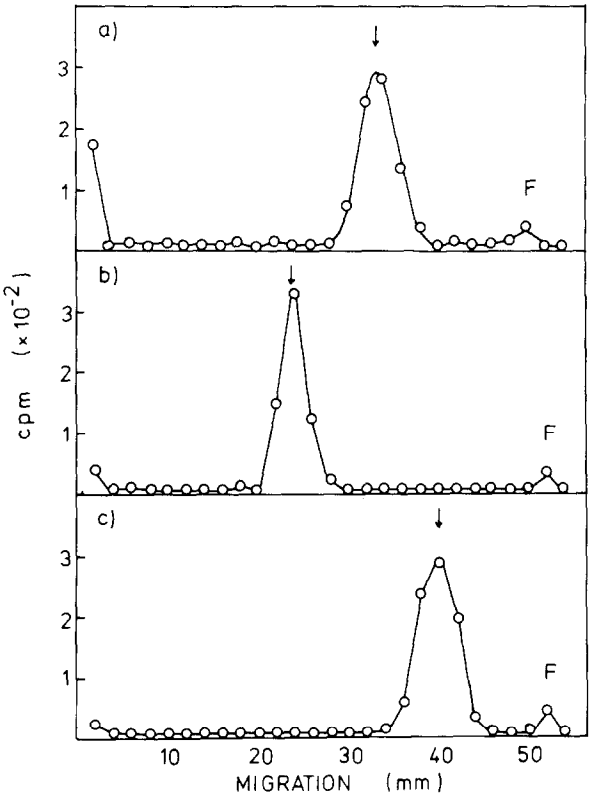

Fig.3. Gel electrophoresis of newly synthesized uteroglobin. The radioactive material eluting from G-75 in the position of uteroglobin (fig.2c) was analyzed by gel electrophoresis (see Methods). The position of uteroglobin is indicated by arrows, and the dye front is labeled 'F'. (a) Gels containing 7.5% acrylamide and run at pH 8.9 [12]; (b) Gels containing 10% acrylamide, 1% SDS, and run at pH 7.0 [14]. Sample treated at 90°C for 5 min in 1% SDS; (c) Same as (b) but with 2% β-mercaptoethanol present during the treatment of the sample.

Table 1
Effect of estradiol and progesterone on uteroglobin synthesis and secretion

Treatment	Protein in uterine fluid (μg/uterus)[a]		[^{3}H]leucine incorporated[b] (cpm/uterus)	
	Total	Uteroglobin	Total secreted proteins	Uteroglobin
None	51	–	30 500	50 (0.2%)
Estradiol-17β (2 days)	170	5 (3%)	160 000	3100 (2.0%)
Estradiol-17β (2 days) + progesterone (4 days)	580	280 (48%)	220 000	96 700 (44.0%)

[a] These values represent the average of 4 determinations in individual uterus. The percentage of uteroglobin was calculated from densitometric tracings of electropherograms (fig.1).

[b] These values represent the average of 2 determinations each including 2 uteri. The total incorporation into secreted proteins was determined by acid precipitation. The percentage of uteroglobin was measured by immunoprecipitation (see Methods). The blank obtained by precipitation with control guinea pig gamma globulin and its rabbit anti-gamma globulin (620 cpm) has been substracted from all values.

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References

[1] Beier, H. N. (1968) Biochim. Biophys. Acta 160, 289–291.
[2] Krishnan, R. S. and Daniel, J. C. (1967) Science 158, 490–492.
[3] Urzua, M. A., Stambaugh, R., Flickinger, G. and Mastroianni, L. (1970) Fertil. Steril. 21, 860–865.
[4] Arthur, A. T. and Cowan, B. D. (1972) Fertil. Steril. 23, 85–92.
[5] Beato, M. and Baier, R. (1975) Biochim. Biophys. Acta 392, 346–356.
[6] El-Banna, A. A. and Daniel, J. C. (1972) Fertil. Steril. 23, 105–114.
[7] Krishnan, R. S. (1971) Experientia 27, 955.
[8] Kirchner, C. (1972) Fertil. Steril. 23, 131.
[9] Bullock, D. W. and Connell, K. M. (1973) Biol. Reprod. 9, 125–132.
[10] Arthur, A. T. and Daniel, J. C. (1972) Fertil. Steril. 23, 115–122.
[11] Krishnan, R. S. (1970) Arch. Biochem. Biophys. 141, 764–765.
[12] Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–413.
[13] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[14] Weber, K. and Osborne, M. (1969) J. Biol. Chem. 244, 4406–4414.
[15] Kay, E. and Feigelson, M. (1972) Biochim. Biophys. Acta 271, 436–441.