PREGNANT MARE SERUM GONADOTROPIN EXHIBITS HIGHER AFFINITY FOR LUTROPIN THAN FOR FOLLITROPIN RECEPTORS OF PORCINE TESTIS

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1. Introduction

Pregnant mare serum gonadotropin (PMSG) is a glycoprotein hormone secreted by the endometrial cup after day 36 of gestation [1]. It exhibits both lutropin (LH) and follitropin (FSH) activities, with LH/FSH activity ratios ranging from 0.5–10 [2–5]. The association equilibrium constants (K_a) of the interaction between PMSG and the LH and FSH gonadal receptors have not yet been determined directly, in a given species, by in vitro binding studies. In this report, the affinity of PMSG for the LH and FSH receptors in porcine testis has been estimated by determination of the binding inhibition potency of the hormone in homologous in vitro radioligand–receptor assays for porcine LH and FSH. We also measured directly the equilibrium association constant (K_a) of ^{125}I-labelled PMSG for the porcine gonadal LH and FSH receptors, and compared the results with the K_a values of the reaction of porcine LH and FSH with their own receptors.

It appeared that in the porcine species, PMSG exhibited higher affinity for the LH than for the FSH testis receptors.

2. Materials and methods

Highly purified PMSG was a gift from Dr D. N. Ward. Highly purified porcine follitropin (pFHS) and porcine lutropin (pLH) were prepared as in [6,7].

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pFSH, pLH and PMSG were enzymatically iodinated by a modification [8] of the method [9].

The homologous radioligand–receptor assay for porcine FSH (pFSH) has been described [8]. Identical conditions were used for the porcine LH (pLH) homologous receptor assay. All studies were performed with the 27 000 X g sedimentation fraction of immature porcine testis homogenate [8].

The non-specific binding was determined in the presence of an excess of cold hormone; this value was subtracted from the total bound radioactivity; with labelled pLH, pFSH and PMSG, the non-specifically bound radioactivity did not exceed 2–3% total radioactivity present in the incubation media. The maximum binding activity of the labelled hormones was determined by incubating a small amount of the tracer preparations with an excess of receptor sites; the specifically bound hormone was expressed as % total radioactivity; the values obtained with labelled pLH, pFSH and PMSG were 30%, 35% and 25%, respectively.

Since PMSG [10] as well as pLH and pFSH were of the highest purity [6,7], FSH and LH activities of PMSG could be determined on a molar basis. Equilibrium association constants (K_a) of pLH, pFSH and PMSG for the gonadal receptors were determined from binding inhibition curves as well as from saturation experiments in which increasing concentrations of labelled hormone were incubated with a constant amount of receptor fraction. No significant difference between the results obtained by both methods was observed. Experimental conditions were as in [8]. The binding data were transformed into Scatchard plots with corrections for the maximum binding

activity of the labelled hormone [11]. The specific activities of each labelled hormone were determined by self-displacement in the corresponding radioligand–receptor assay [11]. Linear Scatchard plots were analyzed with the Scatchard model of a single order of independent binding sites [12]. Upward concave Scatchard plots were fitted with the equation describing the interaction of 1 ligand with 2 independent orders of sites [13]. All computations were performed using the MLAB program [14,15] running on a Digital PDP-10 computer.

3. Results and discussion

Binding inhibition curves of ^{125}I-labelled pFSH and ^{125}I-labelled pLH by pLH, pFSH and PMSG are shown on fig.1. 50% inhibition of ^{125}I-labelled pFSH binding was obtained with 3.3 ng pFSH or 38 ng PMSG, while pLH was completely ineffective. Taking into account the respective molecular weights of pFSH [7] (34 000) and PMSG [16] (53 000), FSH-activity of PMSG amounts to 14% that of pFSH. Figure 1 also shows that 4.7 ng pLH or 13.5 ng PMSG led to 50% inhibition of ^{125}I-labelled pLH binding. On a molar basis, the LH-activity of PMSG is 68% that of pLH, about 5-times higher than its molar FSH-activity.

Scatchard plots derived from binding inhibition curves obtained by incubating ^{125}I-labelled PMSG

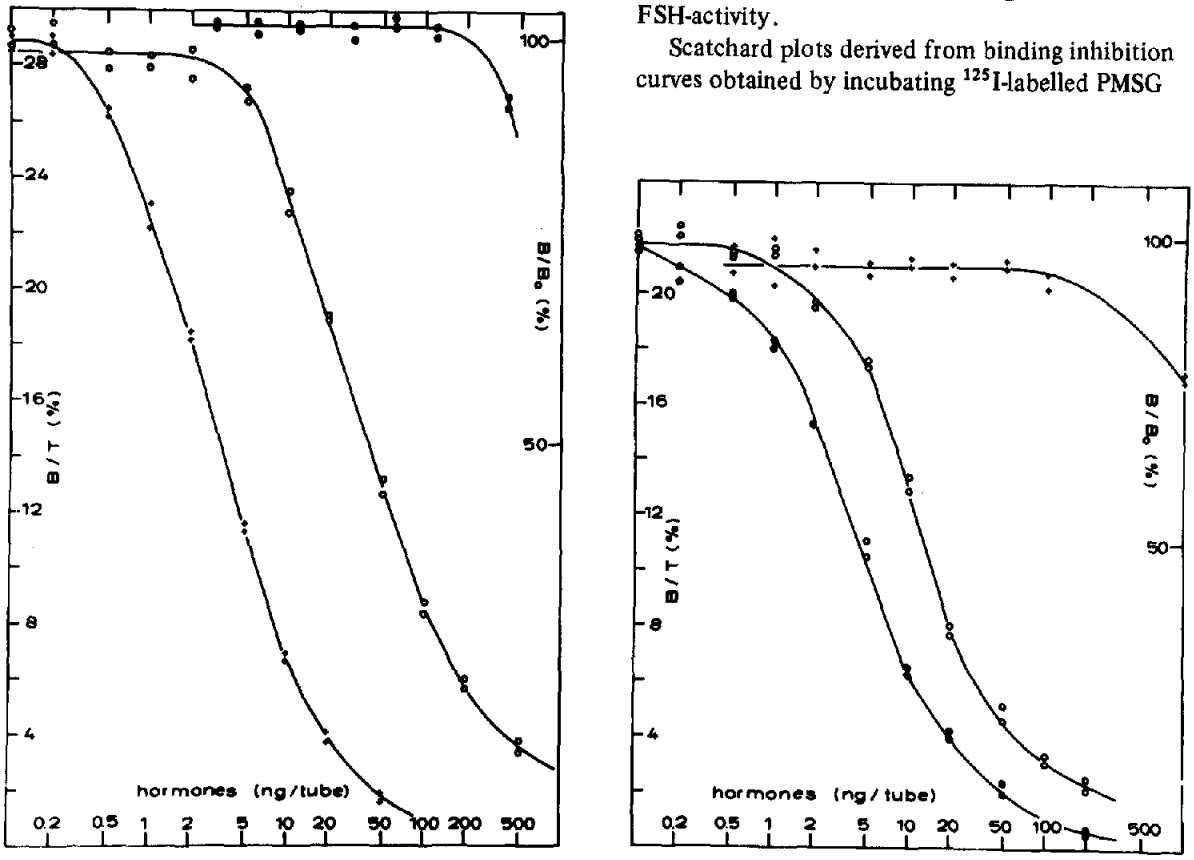

Fig.1. Binding inhibition of ^{125}I-labelled pFSH (left) and ^{125}I-labelled pLH (right) from porcine testis membranes, obtained by pFSH (+——+), pLH (•——•) and PMSG (○——○). 10 000 cpm enzymatically labelled ^{125}I-labelled pFSH or ^{125}I-labelled pLH were incubated for 18 h with increasing amounts of unlabelled hormone and partially purified plasma membranes from porcine testis homogenate (27 000 × *g* sedimentation fraction) [6]. Incubation were performed for 18 h at 24°C in total vol. 250 μl, in 0.05 M Tris buffer, pH 7.5. Particle-bound and free hormone were separated by filtration on Millipore filters (0.45 μm pore size); the non-specifically bound radioactivity was determined in the presence of a 1000-fold excess of unlabelled hormone and subtracted from the total bound radioactivity.

with increasing concentrations of PMSG showed significant upward concavity. This was interpreted as reflecting the presence in the testis fraction of a least 2 classes of binding sites for PMSG; the K_a of the high affinity sites was of 4.7 (± 0.7) 10^{10} M^{-1} and its concentration, 104 (± 11) pM; the K_a of the low affinity sites was of the order of 10^9 M^{-1}, but the precision of the estimate was low. Such data suggested that PMSG interacted with LH and FSH receptors independently and that these receptors exhibited different affinities for the hormone. It is likely, that as in the rat testis [17,18], the LH and FSH receptors in the porcine testis are located on the Leydig cells and the Sertoli cells, respectively.

In order to measure precisely and separately the binding properties of PMSG for the LH and FSH receptors we studied the binding of ^{125}I-labelled PMSG in the presence of saturating amounts of unlabelled pFSH and pLH, respectively. In each case linear Scatchard plots were observed (fig.2). The corresponding K_a and Q values are shown on table 1 together with those of pLH and pFSH for their own receptors. The affinity of PMSG for the LH receptors is 5-times higher than for the FSH receptors in the porcine testis. When K_a values of PMSG for each type of sites are compared to those of pLH and pFSH for their own receptors, its appears that the affinity constant of PMSG for the LH recep-

Table 1
Equilibrium association constants (K_a) and binding sites concentrations (Q) for pLH, pFSH and PMSG in porcine testicular binding fraction (200 μg protein/250 μl)[a]

	K_a (10^{10} M^{-1})	Q (pM)
pFSH	2.1 ± 0.2	132 ± 7
pLH	3.1 ± 0.9	115 ± 27
PMSG (+ pFSH)[b]	2.5 ± 0.2	131 ± 6
PMSG (+ pLH)[b]	0.50 ± 0.05	70 ± 5

[a] K_a and Q are derived from equilibrium binding data as described in the text. Each parameter is shown with the standard error on its estimation computed by the least square analysis program [14,15]. Each curve consisted of 12–16 experimental points

[b] K_a and Q values obtained by incubating increasing amounts of ^{125}I-labelled PMSG with a constant amount of testis binding fraction, in the presence of a large excess (100 ng/tube) of unlabelled pFSH or pLH

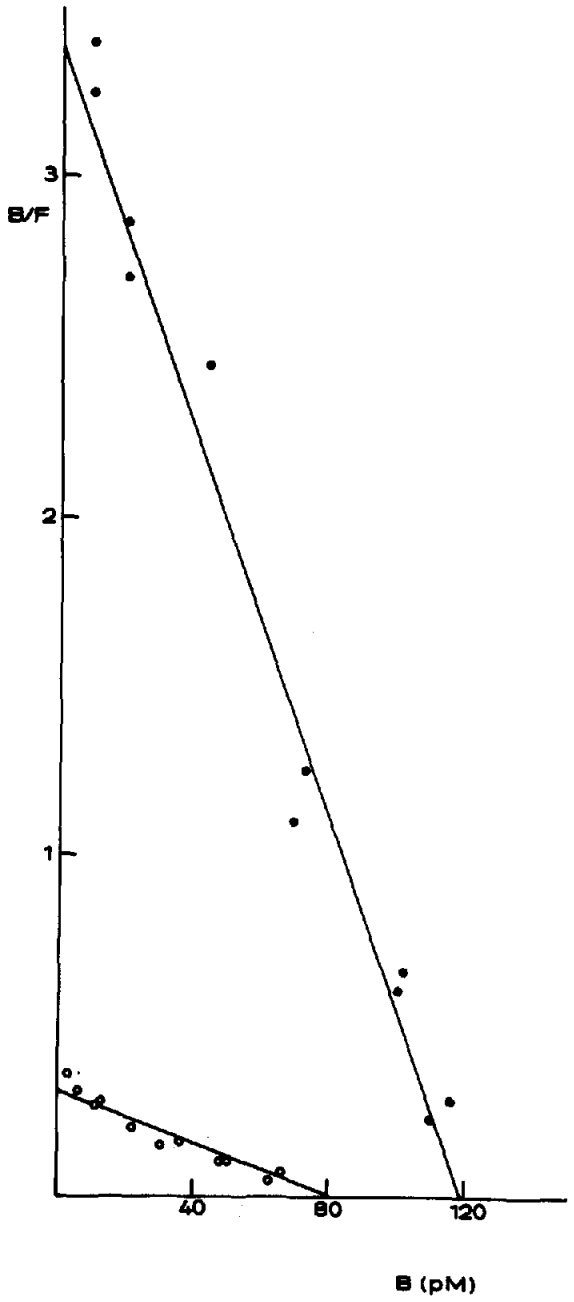

Fig.2. Scatchard plots of saturation curves obtained by incubating increasing concentration of ^{125}I-labelled PMSG with porcine testis membranes in the presence of 100 ng unlabelled pFSH (•—•) or pLH (○—○) per tube. Incubation conditions were as described in the legend of fig.1. PMSG was labelled by the lactoperoxidase method with ^{125}I [6]. Data were analyzed as described in the text.

tor sites is about 80% that of pLH while its affinity for the FSH receptor sites is only 25% that of pFSH. On this basis the LH/FSH activity ratio of PMSG is thus equal to 3. This values is in agreement with that (5) determined by tracer binding inhibition (fig.1).

We have also observed that the binding reaction of tracer amounts of ^{125}I-labelled PMSG with the testis receptor preparation was inhibited by unlabelled pLH but not significantly affected by pFSH (data not shown).

This observation is explained by the fact that tracer amounts of ^{125}I-labelled PMSG will bind predominantly to the LH receptor, because the affinity of the hormone is higher for the LH than for the FSH receptors (see above).

In vitro, PMSG exhibits thus a higher affinity for LH than for FSH porcine testis receptors, while the affinities of LH and FSH for their own receptors are equivalent. This result contrasts with the current concept of PMSG being essentially a follicle stimulating factor in vivo [5,16]. Such a discrepancy might be due to preferential degradation of the LH activity of PMSG in vivo relative to its FSH activity. It might also result from a variability of the LH/FSH activity ratio of PMSG among different species. To answer this question, studies similar to that presented here should be undertaken with other homologous in vitro systems, and the data should be correlated with the in vivo activities of the hormone.

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