The influence of pituitary and gonadal hormones on serum levels of pregnancy-associated murine protein-1

J. Hau1, A. A. Gidley-Baird2, B. Teisner3, P. Svendsen1 and J. G. Westergaard4

Biomedical Laboratory1, University of Odense, Denmark,
Department of Veterinary Physiology2, University of Sydney, Australia,
Institute of Medical Microbiology3, University of Odense, Denmark,
Institute of Obstetrics and Gynaecology4, University of Odense, Denmark.

Abstract. Hypophysectomy of female mice resulted in the disappearance of pregnancy-associated murine protein-1 (PAMP-1) from the circulation within a week. Maintenance of physiological levels of oestrogen, progesterone, testosterone, LH, FSH or prolactin was not sufficient to maintain a normal PAMP-1 level in the hypophysectomized animals. However, administration of oestrogen in large doses to adult male mice with undetectable levels of circulating PAMP-1 caused PAMP-1 to appear in the blood. Testosterone treatment of females inhibited the PAMP-1 synthesis.

Pregnancy-associated murine protein-1 (PAMP-1) (M.W. 150 000) was first described by Hau et al. (1978) in serum of pregnant and non-pregnant female mice but not in serum of adult males. The protein shows partial identity with human pregnancy zone protein, (PZP, α2-PAG), (Hau et al. 1981), and similar to PAMP-1 the serum concentration of PZP shows pronounced inter-individual variation (Folkersen et al. 1981). Both proteins are heterogeneous glycoproteins (Hau et al. 1982a) and they are present intracellularly in the liver and show affinity to the trophoblastic surface exposed to maternal blood (Chemnitz et al. 1982).

The synthesis of human PZP can be stimulated by oestrogen-containing oral contraceptives (Beckman et al. 1971) but PZP and oestriol levels are negatively correlated during pregnancy (Westergaard et al. 1982). Castration of adult male mice results in the appearance of PAMP-1 in circulation after which administration of testosterone inhibits the PAMP-1 synthesis. Ovariectomy results in a very slow decrease in the PAMP-1 concentration (Hau et al. 1982b). These findings suggest a hormonal control of the PAMP-1/PZP level but do not indicate that oestrogens are the direct inducer of the synthesis of these proteins. The present study examines the result of hypophysectomy and hormonal replacement therapy on the PAMP-1 serum levels and the effect of administration of oestrogen to male mice and androgen to female mice.

Materials and Methods

Animals
Experiments were performed with outbred mice of the stocks Cmb:QS and Bom::NMRI. Blood samples were obtained between 09:00 and 11:00 h by periorbital puncture under light diethyl ether anaesthesia. Hypophysectomy was performed using the Bindon (1969) modification of the technique of Lamond & Emmens (1959) under Avertin anaesthesia.

Replacement therapy with pituitary and gonadal hormones was performed using 2-day-pregnant outbred Cmb:QS mice aged 10 weeks. The mice were hypophysectomized on Day-2 of pregnancy in order to synchronize their hormone levels. A pre-operation serum
sample was taken 6 h prior to operation. Replacement injections were given on the evening of Day-2 and morning and evening of Day-3. The mice were killed on the morning of Day-4. The replacement groups were given LH (10 µg/injection) FSH (10 µg/injection), prolactin (10 µg/injection), progesterone (1 mg/injection), oestradiol-17β (10 µg/injection) testosterone (100 µg/injection), gelatine and oil. Protein hormones were given sc in 15% gelatine vehicle and steroid hormones in sesame seed oil. All injected doses were given in 0.1 ml of vehicle, and have previously been demonstrated adequate to maintain physiological levels (Gidley-Baird 1981).

Quantitative rocket immunoelectrophoresis
Rocket electrophoretic quantification of PAMP-1 was performed as previously described (Hau et al. 1978). The amount of PAMP-1 in a serum from mice at 18 and 19 days of gestation (N = 85) was arbitrarily assigned a value of 100 arbitrary units (AU) per ml and used as the master standard. Rocket electrophoretic quantification of murine albumin was performed using an antiserum supplied by the Protein Laboratory, Copenhagen.

The inter-assay coefficient of variation for PAMP-1 and albumin measurements were 5% and 3%, respectively.

Results

PAMP-1 serum levels following hypophysectomy
Hypophysectomy of non-pregnant female outbred Bom:NMRI mice resulted in the disappearance of PAMP-1 within 1 week. Sham-operated animals served as controls (Fig. 1). The serum albumin level was unaffected by hypophysectomy during the study period. PAMP-1 was not detectable in serum prior to or following hypophysectomy of male mice.

The percentual changes in the PAMP-1 levels in the animals treated with hormones after hypophysectomy are shown in Table 1. None of the hormones injected appeared to be able to counteract the fall in PAMP-1 serum level caused by hypophysectomy.

Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in serum PAMP-1 (%)</th>
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<tbody>
<tr>
<td></td>
<td>Prior to operation</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
<td>100</td>
</tr>
<tr>
<td>Progesterone</td>
<td>100</td>
</tr>
<tr>
<td>Testosterone</td>
<td>100</td>
</tr>
<tr>
<td>LH</td>
<td>100</td>
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<tr>
<td>FSH</td>
<td>100</td>
</tr>
<tr>
<td>Prolactin</td>
<td>100</td>
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<tr>
<td>Oil (control)</td>
<td>100</td>
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<tr>
<td>Gelatine (control)</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1.
Changes in PAMP-1 serum level following hypophysectomy and sham operation of outbred NMRI virgin female mice. Values represent means ± SEM for 6 samples at each point.
Stimulation of PAMP-1 in adult male mice by administration of oestradiol-17β

Ten adult male Bom:NMRI mice were treated sc with oestradiol-17β in peanut oil daily for 24 days. During the initial 10 days the dose was 200 μg in 0.1 ml after which the dose was doubled for the remaining period. Prior to the treatment PAMP-1 was undetectable in all mice, on Day-6 of the treatment the PAMP-1 level was 2 ± 2 AU/ml, and on Day-24 the PAMP-1 level had increased to 8 ± 2 AU/ml. It was not possible to increase the PAMP-1 serum level in non-pregnant female mice using a similar treatment with oestradiol-17β.

PAMP-1 serum concentration following testosterone treatment of female mice

Ten non-pregnant adult female mice were treated sc with 0.3 mg testosterone propionate every second day for 8 days, followed by 0.5 mg testosterone propionate every second day until Day-30. Serum levels of PAMP-1 decreased to less than 0.5 AU/ml at Day-23 (Fig. 2).

Discussion

The maternal serum concentration of pregnancy-associated murine protein-1 (PAMP-1) increases during pregnancy, maximum levels being reached at mid-pregnancy. The hormonal regulation of the protein is uncertain. It has previously been demonstrated that the protein is undetectable in serum of adult male mice (Hau et al. 1978) but present during puberty in serum of juvenile males and following castration of adult male mice (Hau et al. 1982b). PAMP-1 is present in serum of female mice and the concentration decreases slowly following ovariectomy (Hau et al. 1982b). Testosterone was found to depress the level of PAMP-1 in circulation (Hau et al. 1982b). These observations indicate that oestrogen and testosterone are involved in the regulation of the PAMP-1 level in serum, but they do not suggest a dose-response relationship between oestrogen and PAMP-1, since ovariectomy results in a slight decrease in serum concentration only and PAMP-1 levels are not significantly influenced by administration of oestradiol to female mice. The present results, however, demonstrate that PAMP-1 synthesis was induced in males by administration of oestradiol. Testosterone was found to inhibit the PAMP-1 synthesis in females and hypophysectomy of females resulted in a disappearance of PAMP-1 from circulation, whereas the serum albumin levels were unaffected during the period studied. Maintenance of physiological doses of oestrogen was not able to counteract the inhibition of the PAMP-1 synthesis and neither was administration of physiological doses of progesterone, FSH, LH, prolactin or testosterone.
Oestrogen does thus not seem to be the sole inducer of PAMP-1 and it seems more likely that the protein is regulated by a more complex mechanism in which testosterone inhibits the protein synthesis.

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References


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