Effects of growth hormone and hypophysectomy of pregnant rats on serum concentrations of pregnancy-associated murine protein-1

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Summary. Continuous infusion of bovine GH to hypophysectomized non-pregnant rats increased serum concentrations of pregnancy-associated murine protein-1 (PAMP-1) to the levels of adult female rats and pregnant rats. Serum concentrations of PAMP-1 were followed from Day 16 of gestation until 3 days after parturition in hypophysectomized (on Day 14 of gestation) and intact pregnant rats. In the intact pregnant rat there was a decrease in PAMP-1 values from Day 16 until delivery. The serum concentrations of PAMP-1 in hypophysectomized pregnant rats were similar to those in intact pregnant rats before parturition, but PAMP-1 concentrations decreased markedly after parturition in the hypophysectomized rats.

We suggest that the serum concentrations of PAMP-1 can be maintained without pituitary GH in late pregnancy, while serum values of PAMP-1 in non-pregnant rats is dependent upon a continuous secretion of pituitary GH.

Keywords: pituitary; hypophysectomy; pregnancy; growth hormone; pregnancy-associated murine protein-1; rat

Introduction

Pregnancy and sex steroids are known to affect the serum concentrations of several liver-derived proteins in man as well as in the rat. The human pregnancy associated α_2 -glycoprotein (α_2 -PAG) and pregnancy-associated murine protein-1 (PAMP-1) in the mouse and the rat are examples of major pregnancy-associated proteins (Folkersen *et al.*, 1981; Hau *et al.*, 1982a; Carlsson-Bostedt *et al.*, 1987). The serum concentration of PAMP-1 is increased by oestrogens (Hau *et al.*, 1982a). During pregnancy serum concentrations of α_2 -PAG in man and PAMP-1 in the mouse are increased. However, the serum concentration of PAMP-1 in mice decreases from mid-pregnancy until term, while α_2 -PAG concentrations remain elevated throughout gestation (Hau *et al.*, 1978, 1982b).

Several studies have indicated an indirect effect of gonadal steroids on the liver via the hypothalamo-pituitary axis affecting the secretory pattern of growth hormone (GH) (Jansson *et al.*, 1985; Edén *et al.*, 1987). In the male rat, GH is secreted in regular episodes with very low levels between peaks, whereas in females GH is secreted in frequent, irregular peaks and with higher baseline levels (Edén, 1979; Jansson *et al.*, 1985; Clark *et al.*, 1987). PAMP-1 has been shown to be unaffected by oestrogen treatment of hypophysectomized females, but increased to the levels of intact females after continuous infusion of human GH, i.e. mimicking the female secretory pattern of GH. In contrast, there was no effect of human GH given intermittently. This indicated that

the secretory pattern of GH could be of importance for the sex differences in PAMP-1 serum concentrations (Fröhlander *et al.*, 1987; Eriksson *et al.*, 1988).

In man, the secretory pattern of GH changes during pregnancy. High levels of immunoreactive GH without apparent peaks are present during late pregnancy (Eriksson *et al.*, 1989). The GH measured is derived from the placental variant of the hormone, whereas the pituitary secretion of GH decrease during late pregnancy (Frankenne *et al.*, 1988; Eriksson *et al.*, 1989). In the rat, however, there are no indications of a placental variant of GH, although the secretory pattern of GH changes during pregnancy (Carlsson *et al.*, 1990). The observation that serum insulin-like growth factor I (IGF-I) levels in hypophysectomized pregnant rats were maintained, in spite of no detectable serum GH concentration, indicated that the fetal-placental unit can maintain the concentration of IGF-I in the absence of pituitary GH (Daughaday & Kapadia, 1978).

With this background it was of interest to investigate whether the serum concentration of PAMP-1 was affected by hypophysectomy in late pregnancy in the rat. Furthermore, to elucidate whether the previously reported effects of human GH on this protein (Eriksson *et al.*, 1988) were due to the somatotrophic or lactogenic action of human GH, the effects of bovine GH were investigated.

Materials and Methods

Animals. Female Sprague-Dawley rats (outbred) were purchased from Alab Laboratories Ltd, Stockholm, Sweden. The rats were housed under controlled conditions of constant temperature (24–26°C), humidity (50–60%), and a 14 h light:10 h dark cycle (lights on at 05:00 h). Standard laboratory chow (Type R-34, Ewos-Alab, Södertälje, Sweden) and tap water were freely available. Hypophysectomy was performed at 40 days of age and on Day 14 of gestation in pregnant rats and age-matched controls (70 days old) by the standard parapharyngeal approach (Smith, 1930) under combined ketamine (Ketalar®, 67 mg/kg; Parke-Davis, Detroit, MI, USA) and xylazine (Rompun®, 13 mg/kg; Bayer, Lever-Kusen, West Germany) i.p. anaesthesia. Completeness of hypophysectomy was determined at the end of the experiments by inspection of the sella turcica.

The pregnant rats and their controls were housed individually and blood samples (300 µl) were taken from the tip of the tail on 5 different days between 09:00 and 11:00 h. Weight gain was recorded every other day (Table 1). The time for parturition was determined by inspection of the cages 3–4 times daily. At the end of the experiment, i.e. 3 days after parturition, all rats were killed by decapitation and trunk blood was collected. Serum was separated and stored at –20°C until assay.

Table 1. Effect of pregnancy and hypophysectomy (on Day 14 of gestation) of pregnant rats on body weight gain

Treatment	No. of animals	Body weight gain (g/day)
Control	4	1.50 ± 0.11
Hypophysectomized	3	0.07 ± 0.19
Normal pregnant	10	7.61 ± 0.76
Pregnant, hypophysectomized	6	3.84 ± 0.77

Values are means ± s.e.m.

Hormonal therapy. All pregnant and non-pregnant hypophysectomized rats received replacement therapy with L-thyroxine (Sigma Chemical Co., St Louis, MO, USA) and hydrocortisone phosphate (Solu-Cortef®, Upjohn, Puurs, Belgium). L-Thyroxine (10 µg/kg/day) and hydrocortisone phosphate (400 µg/kg/day) were given as a daily s.c. injection in a volume of 0.2 ml physiological saline at 08:00 h. Hormonal treatment started the day after hypophysectomy. Bovine growth hormone (bGH) was kindly supplied by American Cyanamide Co. (Princeton, NJ, USA). The hormone was diluted in 0.05 M phosphate buffer (pH 8.8), 1.6% glycerol and 0.02% sodium azide (Schlechter *et al.*, 1986) and given as a continuous s.c. infusion from Day 41 to Day 47 by means of osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA, USA). Bovine GH was given at a dose of 1 or 3 mg/kg/day.

Analysis of pregnancy-associated murine protein-1. Serum concentrations of PAMP-1 was quantified by rocket immunoelectrophoresis (Laurell, 1972) in 1% (w/v) agarose (Medelco Ltd, Hågersten, Sweden) in 0.1 M-Tris-barbital buffer, pH 8.8, at 2.5 V/cm for 15 h. Goat anti-rat PAMP-1 was kindly supplied by Dr Jann Hau (Institute of Veterinary Pathology, Royal Veterinary and Agricultural University, Copenhagen, Denmark). The PAMP-1 antibody was diluted 1:20. Samples of 10 μ l were applied in each well and diluted 1:30 and 1:100. All values were expressed as percentage change from a control group of normal female rats.

Analysis of growth hormone and prolactin. Rat GH and rat prolactin concentrations were determined by radioimmunoassay in duplicate samples of serum (10 μ l) using reagents from NIADDK, essentially as previously described (Clark *et al.*, 1987). Hormone concentrations were expressed in ng/ml in terms of the NIADDK standards GH-RP-2 and PRL-RP-3.

Statistical analysis. Statistical methods used were one-way or two-way analysis of variance, followed by Student-Newman-Keuls multiple-range test between individual groups (Woolf, 1968). Values of $P < 0.05$ were considered significant.

Results

Effects of bovine GH

As shown in Fig. 1 there was a marked decrease in PAMP-1 concentrations after hypophysectomy of normal female rats, which was completely reversed by a continuous infusion of bovine GH. The serum concentrations of PAMP-1 in intact females, pregnant rats at Day 19 of gestation, and hypophysectomized rats after treatment with 1 or 3 mg bGH/kg/day were not significantly different.

Fig. 1. Serum concentrations of rat PAMP-1 in age-matched control female rats (female control), hypophysectomized female rats (hypox) and hypophysectomized rats receiving bovine GH at two different doses. All hypophysectomized rats received thyroxine and hydrocortisone therapy. For comparison, the serum concentration at Day 19 of gestation is also presented (pregnant). Values are expressed as % of the mean value of control rats $\pm$ s.e.m. (vertical bars). There were 4–7 rats in each group. ** $P < 0.01$ vs control rats.

Effects of pregnancy and hypophysectomy of pregnant rats

Hypophysectomy of control females and pregnant rats at Day 14 of gestation caused significant reduction of GH concentrations at Day 16 (mean < 7 ng/ml) compared to intact females. The mean serum levels of GH in control females and females after parturition were not significantly different, while the mean serum concentration of GH in pregnant females was significantly elevated (Table 2). Serum concentrations of prolactin were significantly higher 1 day before and 3 days after parturition compared with those of control females, whereas there was no significant difference between serum levels of prolactin in female controls and females sampled 1 day after delivery (Table 3). In hypophysectomized pregnant rats, serum concentrations of prolactin were undetectable (data not shown).

The serum values of PAMP-1 in pregnant rats were not significantly different from those of intact age-matched females during the first part of pregnancy (data not shown). From Day 16 of gestation until term the serum concentrations of PAMP-1 decreased and were still about 50% of the level of age-matched controls 3 days after parturition (Fig. 2). There were no significant differences between the serum concentrations of PAMP-1 in normal pregnant rats and in hypophysectomized pregnant rats 3 days and 1 day before parturition. There was, however, in contrast to intact rats, a marked decrease in PAMP-1 levels after parturition in hypophysectomized rats (Fig. 2).

Discussion

In the present study continuous infusion of bovine GH to hypophysectomized rats induced a marked increase in serum PAMP-1 concentrations, demonstrating that the effect of human GH

Table 2. Effects of pregnancy and hypophysectomy (on Day 14 of gestation) on serum growth hormone concentrations

Treatment	No. of animals	Serum GH† (ng/ml)
Intact controls	4	22.7 ± 8.3
Hypophysectomized	3	4.2 ± 1.4*
Intact, before parturition	10	93.9 ± 23.3**
Intact, after parturition	10	11.7 ± 2.2
Hypophysectomized, before parturition	6	6.2 ± 2.0*
Hypophysectomized, after parturition	6	1.7 ± 0.2**

Values are means ± s.e.m.

†Serum samples were obtained from the tip of the tail for 3 days during pregnancy and 2 days after parturition. For each rat, the mean serum concentrations of GH before and after parturition, respectively, were considered as one observation.

* $P < 0.05$, ** $P < 0.01$ compared with intact controls.

Table 3. Effects of pregnancy and hypophysectomy (on Day 14 of gestation) on serum prolactin concentrations

Treatment	No. of animals	Serum prolactin (ng/ml)
Intact controls	4	2.0 ± 1.2
Hypophysectomized	3	0.3 ± 0.3
Pregnant, 3 days before parturition	9	0.1 ± 0.1
Pregnant, 1 day before parturition	9	29.5 ± 7.2*
Pregnant, 1 day after parturition	9	10.9 ± 3.6
Pregnant, 3 days after parturition	9	48.8 ± 7.2**

Values are means ± s.e.m.

* $P < 0.05$, ** $P < 0.01$ compared with intact controls.

Fig. 2. Effects of pregnancy in intact (normal) and hypophysectomized pregnant rats (hypox) on serum concentrations of PAMP-1. Hypophysectomy was carried out at Day 14 of gestation. Serum concentrations were measured 3 times before (-5, -3 and -1 days) and twice after (1 and 3 days) parturition. Serum values are expressed as % of the mean value of age-matched control females. S.e.m. values are indicated by vertical bars. There were 6–10 observations in each group. Values with different letters are significantly different ($P < 0.05$).

(Eriksson *et al.*, 1988) on serum PAMP-1 levels in the rat is due to a somatotrophic action of the hormone (Postel-Vinay, 1976). Moreover, there was no further increase in PAMP-1 serum values by giving higher doses of GH, indicating that a physiological increase of serum GH concentrations, as in pregnancy (Terry *et al.*, 1977; Carlsson *et al.*, 1990), would have no or small effects on serum PAMP-1 levels. In spite of the high continuous serum concentrations of GH in late pregnancy (Terry *et al.*, 1977; Carlsson *et al.*, 1990), the serum concentrations of PAMP-1 in late pregnancy decreased and were similar to or lower than those of age-matched controls (see Figs 1 & 2), indicating that the serum PAMP-1 concentrations are not solely regulated by GH. This finding is in contrast to that in pregnant mice, which have higher serum concentrations of PAMP-1 throughout gestation, although the serum levels of PAMP-1 decrease in late pregnancy in both species (Hau *et al.*, 1982b). Hypophysectomy of pregnant rats at Day 14 of gestation did not affect the serum PAMP-1 value at Day 18 or 20 of gestation in spite of low immunodetectable concentrations of GH. In contrast to the intact rats, there was a marked decrease in PAMP-1 concentrations in hypophysectomized rats after parturition. This finding again indicates that the serum concentration of PAMP-1 during late pregnancy is maintained by factors other than pituitary GH. Similar results have been obtained with respect to IGF-I activity, which was maintained in hypophysectomized pregnant rats, despite serum concentrations of GH being undetectable (Daughaday & Kapadia, 1978). The concentrations of serum IGF-I in the rat increased to mid-pregnancy followed by a decrease to 20–40% of the level in non-pregnant rats at the end of gestation (Sheppard & Bala, 1986). The time courses of the changes in PAMP-1 and IGF-I serum concentrations suggest that these two proteins may be regulated in a similar way or may influence each other during pregnancy in the rat. A possible candidate for the maintenance of IGF-I and PAMP-1 concentrations in hypophysectomized pregnant rats is chorionic somatomammotrophin, which is known to increase after hypophysectomy of pregnant rats (Daughaday *et al.*, 1979).

In the rat, the concentrations of PAMP-1 decreased to about 10% of the value of normal females 9 days after hypophysectomy (Eriksson *et al.*, 1988). PAMP-1 has a half-life in serum of

26 h in the mouse (Hau *et al.*, 1982b, 1983). The maintenance of PAMP-1 in hypophysectomized pregnant rats could also be explained by a longer half-life of PAMP-1 in serum during pregnancy.

In conclusion, there are different regulatory principles determining the PAMP-1 concentrations in the pregnant and non-pregnant rat. PAMP-1 serum concentrations are regulated by GH in the non-pregnant rat, but in the pregnant rat serum values of PAMP-1 seem to be regulated by hormones or factors other than GH.

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