Placental lactogen administration reverses the effect of low-protein diet on maternal and fetal serum somatomedin levels in the pregnant rat

growth hormone/lactogenic receptor/placenta/insulin-like growth factors/multiplication-stimulating activity

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ABSTRACT Female rats were studied on day 20 of pregnancy after being fed either a 5% lactalbumin (low protein) diet or a 20% lactalbumin (adequate) diet for the last 2 weeks of pregnancy. Rats on the lower intake of protein showed decreased serum levels of rat placental lactogen and reduced numbers of lactogenic receptors in the maternal liver. These changes were accompanied by much reduced serum levels of somatomedins IGF I (insulin-like growth factor) and II (multiplication-stimulating activity, MSA). Infusion of human placental lactogen or human growth hormone into the rats on the low-protein intake during the last 2 weeks of pregnancy partially restored the maternal serum levels of both somatomedins, but only human placental lactogen increased the number of lactogenic receptors on liver cell membranes. It was concluded that protein deficiency may reduce secretion of somatomedins by the liver (or other tissues) of the pregnant rat indirectly through reduction in output of rat placental lactogen by the placenta. In the same experiments, the effect of maternal protein deficiency on fetal development and serum somatomedin levels was examined. Protein deficiency resulted in smaller fetuses and placentas and lower fetal serum levels of IGF I and MSA. Unlike the response in maternal serum levels, the concentration of MSA in the fetal serum increased during infusion of hPL or hGH but the concentration of IGF I did not. This suggests that placental lactogen enters the fetal circulation and affects tissues producing MSA but not those making IGF I. Despite the restoration of MSA levels, fetal and placental weights did not increase when the rats on the protein-deficient diets were treated with human placental lactogen or growth hormone.

Plasma levels of somatomedins (IGFs) increase along with placental lactogen (PL) levels during the latter part of pregnancy in man (1) and the rat (2). There is persuasive evidence that during pregnancy PL substitutes for growth hormone (GH) in regulating the secretion of somatomedins. Thus, hypophysectomy of the rat at midpregnancy does not reduce the serum level of IGF I whereas hypophysectomy of the nonpregnant rat results in a decrease in serum IGF I to undetectable levels (3), which can be restored by administration of ovine PL (4). In confirmation, perfusion of the isolated rat liver with human PL (hPL) stimulates output of somatomedin (5). We have found that pregnant rats restricted in protein intake have low concentrations of rat PL (rPL) in their placenta and sera, as well as depressed serum levels of somatomedin and its carrier proteins (6, 7). There is also failure of the livers of the protein-deficient pregnant rats to show the increase in size and in lactogenic receptor number (7) that normally occurs during pregnancy. To further investigate the relationship of these events, we have now administered hPL and human growth hormone (hGH) by continuous infusion pump to pregnant rats maintained on a protein-deficient diet during the last 2 weeks of pregnancy. We have examined the responses of the somatomedins insulin-like growth factor (IGF) I and II (multiplication-stimulating activity, MSA) in maternal and fetal serum, as well as changes in lactogenic receptor number in the maternal liver.

MATERIALS AND METHODS Materials. High-concentration sodium [125I]iodide was purchased from Amersham and New England Nuclear. Chloramine-T was obtained from Gallard Schlesinger (Carle Place, NY). Lactoperoxidase (60–80 units per mg), human IgG, polyethylene glycol (6000), fraction V bovine serum albumin (A-4503), and RIA grade bovine serum albumin were purchased from Sigma. Pansorbin Staphylococcus aureus protein A in suspension was purchased from Calbiochem—Behring. hPL RIA kits were obtained from Amersham, while hGH RIA kits came from Kallstadt (Austin, TX). Purified rat IGF II (MSA III-2) and rabbit anti-rat MSA antibody were gifts from S. F. Nissley (National Cancer Institute). IGF I (preparation 1-4) was a gift of René Humbel (Zurich, Switzerland). Rabbit anti-human somatomedin C (IGF I) antibody was a gift from Richard Furlanetto (Philadelphia). hPL, ovine prolactin (oPRL) (RIA grade and biological grade), and hGH were provided by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

Experimental Design. Pregnant Sprague-Dawley rats (C-D strain) were obtained from Charles River Breeding Laboratories on day 4 or 5 of pregnancy. They received diets providing either 20% or 5% protein (lactalbumin), the other constituents being similar (7). In a pilot experiment, these two diets were fed from day 6 through day 21 of pregnancy to observe responses of IGF I and MSA to protein intake. Some of the rats on the 5% protein diet were also given 75 µg of hPL daily by subcutaneous minipump (see below) to determine whether this hormone could restore serum somatomedin levels depressed by the low-protein diet. In the main experiment, rats were divided into six groups on day 7 of pregnancy. One group, designated 20P, was allowed ad lib access to the 20% protein diet. A second group (5P) received the 5% protein diet, fed ad lib. A third group

Abbreviations: PL, placental lactogen; rPL and hPL, rat and human PL, respectively; IGF, insulin-like growth factor; MSA, multiplication-stimulating activity (rat IGF II); oPRL, ovine prolactin; GH and hGH, growth hormone and human GH.
(20PF) was pair-fed the 20% protein diet in quantities that were isocaloric to the intake of the 5P group on the same day of gestation. Three additional groups that received the 5% protein diet received implants under ketamine anesthesia of subcutaneous Alzet osmotic minipumps (Alza, Palo Alto, CA) containing either (i) the vehicle, sterile 0.9% saline (group 5VEH), or (ii) hPRL in vehicle at a concentration to deliver 75 μg of hormone/day (group 5PPL), or (iii) hGH in vehicle at a concentration to deliver 50 μg/day (group 5GH). All rats were weighed and then decapitated on day 20 of gestation. Serum samples were collected from dams and fetuses and stored for not more than 3 months at −70°C. The sera from all fetuses in each litter were pooled to provide one sample for assay. Maternal livers and placentas were blotted, weighed, quick-frozen on dry ice, and stored at −70°C.

**Assay for rPL.** A radioreceptor assay (8) was used to estimate rPL levels in maternal and fetal rat serum. Using RIA grade oPRL, 125I-labeled oPRL was prepared by a modification of the lactoperoxidase procedure (9, 10), to yield a specific activity of 60–100 μCl/μg (1 Ci = 37 GBq), purified on a Sephadex G-75 column in 25 mM Tris-HCl, pH 7.4,0.1 M CaCl2,0.2% bovine serum albumin and stored at −20°C, where it was stable for up to 2 weeks. Microsomal membranes, isolated from the livers of day 12 to day 18 pregnant rats (8), were used as the source of lactogenic receptors for the assay. Unlabeled oPRL (biological grade) was used for the standard curve. Untreated serum samples (25 μl) were incubated with 125I-labeled oPRL and 250 μg of membrane protein in a total volume of 0.5 ml overnight at 4°C. The reaction was terminated by addition of 2 ml of ice-cold 25 mM Tris-HCl (pH 7.4)/0.1% bovine serum albumin/10 mM CaCl2 followed by centrifugation at 4°C and aspiration of the supernatant; the percentage of radioactivity in the pellet was measured. Least-squares regression lines were computed for unlabeled oPRL for each assay and rPL concentrations were calculated relative to these (8).

**Measurement of Lactogenic Binding to Maternal Liver Membranes.** Microsomal membranes, isolated from 1-g slices of each maternal liver (8), were incubated as described above with 125I-labeled oPRL in the presence or absence of excess unlabeled oPRL. Binding data are expressed as a percentage of the total radioactivity added that was specifically bound to 250 μg of membrane protein under equilibrium conditions. Membrane protein concentrations were determined by the method of Lowry et al. (11) after solubilization in 1 M NaOH at 100°C for 10 min.

**Assay for Rat Prolactin.** Rat prolactin was measured by RIA using reagents and procedures provided by A. F. Parlow on behalf of the Pituitary Hormone Distribution Program.

**Assays for hPL and hGH.** The concentrations of hPL and hGH were measured in untreated maternal serum samples by specific RIAs using kits according to the instructions of the manufacturer.

**Assays for IGFs.** IGF I and IGF II (MSA) concentrations were determined by separate specific RIAs of individual maternal and fetal sera, after acid gel filtration on Sephadex G-50 to remove serum carrier proteins (12). Both 125I-labeled MSA III-2 and 125I-labeled IGF-I were prepared according to the chloramine-T procedure of Rechler et al. (13) and stored at −20°C prior to use. If used more than 2 weeks after iodination, labeled somatomedins were repurified on a Sephadex G-25 column (1 × 6 cm) equilibrated with RIA buffer.

The MSA RIA was carried out according to the procedure of Moses et al. (14), using a rabbit anti-rat MSA polyclonal antibody and unlabeled MSA III-2 as the standard. The specificity of this antiserum has been reported (14). The IGF I RIA was carried out according to the method of Furlanetto et al. (15), using rabbit anti-human somatomedin C (IGF I) antibody. Pure unlabeled IGF I was used as the standard. In addition, a standard displacement curve was constructed using increasing concentrations of acid gel-filtered normal rat serum for each assay to ensure its linearity with the pure IGF I standard.

**Statistical Analysis.** Data were compared by one-way analysis of variance, differences between individual groups being evaluated by the Newman–Keuls procedure. Graphs show standard errors as vertical bars.

**RESULTS**

Since we had shown previously (7) that a low-protein diet depresses serum rPL levels and total serum somatomedin concentration in the pregnant rat, a pilot experiment was carried out to examine the effect of a low intake of protein from day 6 through day 21 of pregnancy on maternal serum levels of IGF I and MSA separately and whether exogenous PL (hPL) given subcutaneously over the same period could restore these levels toward normal. At the end of pregnancy, animals fed the 20% protein diet had average serum levels of 510 ± 44 ng of IGF I/ml and 405 ± 34 ng of MSA/ml whereas the serum concentrations of these somatomedins for rats on the 5% protein diet were significantly lower—126 ± 46 (P < 0.001) and 43 ± 34 (P < 0.01), respectively. When rats

![FIG. 1. Maternal weight gain (A) from day 7 through day 20 of pregnancy and weights of livers (B) on the 20th day. Groups studied received either the 20% protein diet (20P) fed ad lib (seven rats), the 20% protein diet pair-fed (20PF) with rats on the 5% protein diet (four rats), the 5% protein diet (5P) fed ad lib (seven rats), the 5% protein diet with vehicle (5VEH) delivered by subcutaneous pump (five rats), the 5% protein diet with 75 μg of hPRL (5HPL) injected daily (six rats), or the 5% protein diet with 50 μg of hGH (5GH) injected daily (three rats) from day 7 through day 20. Results represent mean ± SEM.](image-url)
 consuming the 5% protein diet also received continuous subcutaneous infusion of hPL, the serum levels of the two somatomedins were raised to 273 ± 53 and 175 ± 50 ng/ml, respectively, both significantly higher (P < 0.05) than for untreated rats on the 5% protein diet.

This pilot experiment was followed by an experiment in which the effects of infusion with hGH were compared with those of hPL, and the responses of fetal serum somatomedins to diet and to hormonal treatment were also examined. On day 7 of pregnancy, the rats were divided into six groups and were given either the 20% or the 5% protein diet, as follows: (i) the 20% protein diet ad lib (group 20P); (ii) another group on the 20% protein diet were pair-fed (20PF) with rats on the 5% protein diet (5P). The animals fed the 5% protein diet were divided into four groups: (i) one group received only the low-protein diet (5P), and (ii–iv) the other groups received vehicle only (5VEH), hPL (5HPL), or hGH (5HGH) through implanted minipumps.

Pregnant rats fed the 5% protein diet (5P, 5VEH, 5HPL, and 5HGH) during the last 2 weeks of pregnancy (Fig. 1) gained less weight than rats fed the 20% protein diet (20P). The group of rats fed 20% protein but matched calorically to the 5% protein group (20PF) also gained less weight than the controls. Treatment of rats on the low-protein diet with hPL (5HPL) or hGH (5HGH) did not restore weight gain to control (20P) levels. By comparison with the 20P group, liver weight at the end of pregnancy was significantly lower in all other groups, which did not differ among themselves. The placentas of all groups of rats receiving the 5% protein diet weighed significantly less than those of rats on the 20% protein diet (20P), while the rats receiving hGH (5HGH) had placentas that were significantly smaller than those of the other rats on the 5% protein diet (Fig. 2). Finally, although litter size was not consistently affected by the treatments, the weight per fetus (Fig. 2) was reduced both by food restriction (20PF) and by feeding the 5% protein diet with (5HPL and 5HGH) or without (5P) the infused hormones.

The rPL levels in maternal serum (Fig. 3) were significantly reduced by pair-feeding (20PF) and were further reduced by feeding the low-protein diet (5P), an effect that was not altered by pump implantation without hormonal treatment (5VEH). However, infusion of hPL (5HPL) or hGH (5HGH) considerably increased the apparent serum rPL levels of rats fed the 5% protein diet. Since we have confirmed evidence (15) that both hPL and hGH displace oPRL from rat liver membranes under the conditions of the rPL assay used by us, we conclude that rPL levels in the serum of hormone-treated rats do not represent true concentrations. Specific RIA data were used to measure the levels of hPL and of hGH in maternal serum of the infused groups at the end of pregnancy. The apparent hPL concentration was 120 ± 9 ng/ml in the serum of the high-protein group (20P) and 330 ± 110 ng/ml in the hPL-treated group (5HPL), while hGH was 2.4 ± 0.1 ng/ml for the 20P group and 23.3 ± 9.9 ng/ml for the hGH-treated group (5HGH). The demonstration of significant levels of hPL in animals not treated with the hormone indicates that some factor in normal pregnant rat serum, probably rPL, cross-reacts in the assay for hPL, resulting in the high control levels. Fig. 3 also shows that the lactogenic binding capacity of the liver membranes of rats receiving the 5% protein diet (5P and 5VEH) was reduced and that this reduction was reversed by hPL treatment (5HPL) but not by hGH treatment (5HGH). Since the liver was not restored to its normal pregnant size by hPL treatment (Fig. 1), the total population of receptors was only partly restored toward normal by treatment with this hormone (data not shown).

Regarding the somatomedins, as shown in Fig. 4, the lev-
levels of IGF I and MSA in maternal serum were lowered significantly by the 5% protein diet (5P and 5VEH) and were maintained near normal by hPL infusion (5HPL); in addition, hGH treatment (5HGH) also prevented the fall in the maternal levels of these two somatomedins caused by the 5% protein diet.

Fetal blood also was assayed for its content of IGF I and MSA (Fig. 5). In agreement with published observations (16), rat fetal serum MSA levels were severalfold higher than those of maternal serum (Fig. 4)—1050 versus 200 ng/ml. In contrast, fetal rat IGF I levels were much lower than maternal blood levels—225 versus 750 ng/ml. The feeding of the low-protein diet significantly lowered both MSA and IGF I levels in fetal serum. hPL and hGH administration reversed the effect of diet on fetal serum MSA levels but not that on fetal serum IGF-I levels.

In view of the changes in fetal serum somatomedin levels, it was of interest to know whether rPL was present in fetal serum and whether it varied with maternal diet. When fetal serum was tested by the rPL radioreceptor assay, significant amounts of this hormone were detected—from 80 to 28 ng/ml on the 20% and 5% protein diet, respectively. These quantities could not be accounted for by cross-reaction with fetal prolactin, which, in agreement with published evidence, was found by a specific RIA to be present in only small quantities (1.1–3.7 ng/ml fetal serum). On the other hand, the apparent rPL activity in fetal serum could have been due to cross-reaction with fetal GH, which is elevated in fetal serum (17). A notably high level of rPL (260 ng/ml) was found in rats receiving hPL, suggesting that hPL, which cross-reacts in the rPL assay (see above), can traverse the placenta into the fetal circulation and presumably rPL can also do so.

**DISCUSSION**

When rats are made protein deficient during the last 2 weeks of pregnancy, maternal serum levels of rPL and total somatomedin decrease in comparison with rats fed adequate protein (7). This suggests that somatomedin formation by the liver and other tissues during pregnancy may be regulated by PL acting as an analog of growth hormone. The present study confirms this; serum levels of both MSA and IGF I decrease after severe protein deprivation of the pregnant rat and the effect is reversed by infusion of PL. Previous investigators have shown that administration of PL to nonpregnant rats stimulates the release of somatomedin A from the perfused liver (5) and maintains normal serum levels of IGF I in hypophysectomized rats (4), and we have now found that adequate serum levels of PL are needed to maintain maternal somatomedin output during pregnancy. This agrees with earlier observations that hypophysectomy of pregnant rats causes a rise in rPL levels in maternal serum (18) accompanied by maintenance of serum somatomedin levels (3) despite the loss of GH, which regulates serum somatomedin levels in nonpregnant animals (19). The regulatory role of PL in pregnancy is confirmed in humans by the observation that women whose fetuses lack both genes for hPL and whose placentas consequently do not secrete hPL exhibit decreased maternal levels of IGF I (20).

Our findings provide some evidence regarding factors that determine the response of the somatomedins to hPL. The increments in body weight and liver size occurring in the normal pregnant rat were not restored by hPL or hGH administration to malnourished pregnant rats (Fig. 1), presumably because of the restriction on the rate of tissue protein synthesis through insufficient amino acid supply from the diet low in protein. Nevertheless, the stimulation of IGF I
and MSA levels caused by hPL administration to rats on the 5% protein diet was accompanied by increased numbers of hepatic lactogenic receptors. Administration of hGH also caused an increase in serum somatomedin levels in the malnourished rats that was not accompanied by an increase in hepatic lactogenic receptors; presumably specific GH receptors (not measured) increased after hGH administration. The behavior of maternal somatomedin levels appears to correlate best with the number of the hepatic lactogenic receptors, notably in the case of the pair-fed controls (20PF), which show a large reduction in serum levels of rPL (Fig. 3), but not in levels of IGF I or MSA (Fig. 4) or in lactogenic receptors (Fig. 3).

The effects of malnutrition and hormonal status on fetal somatomedins were found to differ from those in the dams. The relative concentrations of the two rat somatomedins are different in mother and fetus (Figs. 4 and 5). In the maternal serum of well-nourished pregnant rats, MSA is half the level of IGF I whereas fetal serum contains 5 times more MSA than IGF I. When the rats were fed the diet low in protein, fetal serum levels of both MSA and IGF I declined but only MSA levels were restored to normal concentrations by infusion of hPL or hGH (Fig. 5). This difference in fetal response makes the maternal somatomedins unlikely sources of the changes in fetal levels unless there are specific differences in the placental transport of IGF I and MSA. Further, there is ample evidence that some fetal rat tissues produce large amounts of MSA (21, 22), while IGF I has not been detected in fetal fibroblasts (22). Accordingly, the administered hormones hPL and hGH must either penetrate the placental barrier and affect fetal tissue somatomedin production directly or else act indirectly through a placental mechanism. The former seems the more likely, since PL has been found in human (23) and sheep (24) fetal blood, and in our experiments fetal serum PL levels increased when hPL was given to the malnourished mothers. The fetal tissues that synthesize and secrete MSA in response to PL (or GH) have not been identified. MSA release from rat fetal fibroblasts in tissue culture is unaffected by addition of hGH to the medium but responds to addition of ovine PL (22). In our study (Fig. 5), administration of hGH as well as of hPL to malnourished rats raised fetal MSA levels, suggesting that some somatomedin-secreting fetal tissues other than fibroblasts must be sensitive to GH.

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