Lactogenic actions of different growth hormone preparations in pregnant and lactating rats

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Abstract

We studied the capacity of different GH preparations, natural human (h)GH, recombinant hGH (rhGH), rat (r)GH, ovine (o)GH, bovine (b)GH and porcine (p)GH, and ovine prolactin (oPRL), to stimulate lactogenesis in ovario-hysterectomized pregnant rats or intact lactating rats treated with bromocriptine (BC). Ovario-hysterectomy (OVX-HYS) performed at 0800 h on day 19 of pregnancy induced lactogenesis, i.e. increases in mammary casein and lactose and positive response to the oxytocin test, 28 h later. Lactogenesis was prevented by treatment with BC (1·5 mg/kg) immediately after surgery (OVX-HYS-BC). The hormones were given at doses of 0·25 or 0·5 mg/rat (except rhGH given only at 0·5 mg/rat) at 1200 and 2000 h on day 19. Casein was increased by both doses of oPRL and hGH, rhGH and 0·25 mg oGH, and lactose by both doses of oPRL, rhGH and 0·25 mg rGH. The other GH preparations had no effect. The oxytocin test demonstrated the presence of milk in the mammary tissues of the OVX-HYS rats and in the OVX-HYS-BC plus oPRL (0·25 and 0·5 mg) or rhGH-treated groups.

Injection of BC to pregnant rats at 2000 h on day 20 and at 0800 h on day 21 decreased litter growth on the first 4 days postpartum. Two-thirds of the litters resumed growth after day 4, indicating the recuperation of milk production, while the rest never recuperated. Serum prolactin in BC-treated rats was reduced until day 4 postpartum. On day 6 the rats which had recuperated had normal values, while those which had still not recuperated had lower values. BC-treated rats were injected s.c. with 0·25 mg each of oPRL, hGH, rGH, oGH, bGH or pGH, or 0·25 or 0·5 mg rhGH/rat, immediately postpartum and 12, 24 and 36 h later. hGH and 0·5 mg rhGH induced levels of milk production similar to controls except on day 3. oPRL and rhGH (0·25 mg), induced a partial reversion of the effect of BC. rGH and oGH had a slight effect on days 1 and 2 and all the litters resumed growth on day 7. In contrast, pGH and bGH were inactive.

The affinity of hGH for the prolactin receptor, measured as displacement of ^{125}I-labelled oPRL binding to crude liver membranes, was comparable with that of oPRL. While rhGH was ten times less active than oPRL, rPRL was 100 times lower and all the other GH preparations had at least 10^4 times lower capacity to displace ^{125}I-labelled oPRL.

These results indicate that both natural and recombinant hGHs are potent inductors of milk synthesis in pregnant or lactating rats, most probably due to their actions at the level of the prolactin receptor. rGH and oGH have a partial action, while pGH and bGH seem to be inactive. The actions of non-human GHs may be explained by their somatogenic properties exclusively, and indicate that GH may play a role in the optimization of milk production during lactation and an accessory role in the induction of lactogenesis in pregnant rats.

Journal of Endocrinology (1994) **142,** 535–545

Introduction

Growth hormone (GH) and prolactin, as well as their respective receptors, have a great degree of genetic similarity, since they are thought to derive from a common ancestor through gene duplication (Kawauchi & Yasuda 1988, Kelly *et al.* 1991). This similarity extends to their functions, which show some degree of overlap. Perhaps the most striking example of this, is the great capacity of human GH (hGH) to bind to lactogenic receptors and to mimic the actions of prolactin in several of its target tissues, for example, the mammary gland (Forsyth *et al.* 1965, Posner 1976, Chene *et al.* 1986). This action is evident in various species but not in human tissues, where hGH seems to have mostly somatogenic properties. Conversely, prolactin also can show some of the actions typically ascribed to GHs, such as growth regulation (Esquifino *et al.* 1987, Crowe *et al.* 1991).

In most species of domestic ruminants, GH is more important than prolactin in inducing increases in milk production during lactation, although this action is due to an effect in nutrient partitioning, increasing the flow of

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0022–0795/94/0142–0535 $08.00/0

nutrients to the mammary gland, rather than a direct lactogenic effect stimulating milk synthesis (Cowie 1969, Tucker 1988). It has been shown that GH plays a role in stimulating milk production in lactating rats (Madon *et al.* 1986, Flint *et al.* 1992). The stimulating action of GH on lactation may be mediated through insulin-like growth factors (IGFs), whose titres were depressed by treatment with GH antiserum and normalized after replacement treatment with bovine GH (bGH) (Flint *et al.* 1992). By contrast, Flint *et al.* (1992) were unable to achieve any response in milk production to exogenous IGF-I, suggesting that IGF-I alone does not mediate these actions of GH. IGFs have been shown to stimulate prolactin-induced milk synthesis in culture (Houdebine *et al.* 1985) as well as mammary mitogenesis (Forsyth 1989). Furthermore, both IGF-I and an amino-terminally shortened form of IGF-I (Des-(1–3)IGF-I) were capable of substituting for the pituitary gland in oestrogen-induced mammary development (Ruan *et al.* 1992).

It is apparent from current studies that high concentrations of GH can cause lobulo-alveolar development and casein expression on mouse mammary tissue, similar to that produced by ovine prolactin (oPRL). However, IGF-I did not substitute for prolactin or GH in tissue maintenance (Plaut *et al.* 1993). On the other hand, GH receptor mRNAs have been recently described in the mammary gland from rabbits and rats (Jammes *et al.* 1991, Tiong & Herington 1991) and GH has some direct action on rat mammary epithelial cells, mainly as a mammogenic and mitogenic factor (Kleinberg *et al.* 1990). More importantly, recent studies (Feldman *et al.* 1993) have provided evidence that, for the process of mammary development, GH acts through the GH receptors, and strongly support the hypothesis that the GH receptor plays a central and primary role in mammary development. According to the authors, the mechanisms of action of GH does not involve a lactogenic receptor. Furthermore, rat placentae produce GH-like proteins (Ogilvie *et al.* 1990), which may play a role in governing maternal metabolism during pregnancy, along with or replacing pituitary GH (Frankenne *et al.* 1988).

In the present work we have studied the capacity of GH preparations from different species (rat, human, ovine, porcine and bovine) to replace placental lactogen or prolactin in the induction of lactogenesis in pregnant rats and in the maintenance of postpartum lactation. We have compared the results obtained with the binding capacity of the same GH preparations to the rat liver lactogenic receptor. Partial results from this study have been published in abstract form (Deis & Jahn 1988, Carón *et al.* 1992).

Materials and Methods

Animals

Virgin female rats, 3–4 months old (200–220 g) bred in our laboratory and originally of the Wistar strain, were used. The rats were kept in a light (lights on 0600–2000 h)- and temperature (22–24 °C)-controlled room; rat chow (Nutric, Córdoba, Argentina) and tap water were available *ad libitum*. Vaginal smears were taken daily. Rats were caged individually with fertile males on the night of pro-oestrus, and the presence of spermatozoa was checked in the vaginal smear the following morning. This day was designated day 0 of pregnancy. In our laboratory most rats usually give birth in the night between days 21 and 22, although approximately 25% deliver in the afternoon of day 21. The day of parturition was considered to be day 0 of lactation.

In the first experiment, pregnant rats were ovariectomized and hysterectomized under ether anaesthesia and injected s.c. with 1·5 mg bromocriptine/kg (BC, a generous gift from Sandoz, Buenos Aires, Argentina) dissolved in saline:ethanol (7:3) between 0800 and 0900 h on day 19 of pregnancy (OVX-HYS-BC). Groups of rats were injected s.c. with two doses of 0·25 or 0·5 mg oPRL/rat (ovine NIDDK-oPRL-19 AFP-9221A), rat GH (rGH; rat NIDDK-rGH-B-12 AFP-10478C), ovine GH (oGH; ovine NIDDK-oGH-15 AFP-7694C), porcine GH (pGH; porcine USDA-pGH-B-1 AFP-5100), bGH (bovine USDA-bGH-B-1 AFP-5200), hGH (human NIDDK-hGH-B-1 AFP-9755A) (all generously provided by NIDDK, Bethesda, MD, USA) or recombinant hGH (rhGH; Saizen, generously provided by Serono, Buenos Aires, Argentina), dissolved in polyvinylpirrolidone (25%) in water (1 mg/ml) at 1200 and 2000 h on the same day. Control OVX-HYS-BC rats were injected with the vehicle. An additional group of rats was ovariectomized and hysterectomized as above, but the injection of BC was omitted (OVX-HYS) and a group of sham-operated rats was included as intact controls. The rats were killed by decapitation at 1200 h on day 20 of pregnancy. The whole abdominal and inguinal mammary glands were quickly excised, small portions were separated to determine the presence of milk by a modified oxytocin test (Deis 1968), and the rest frozen at −70 °C until casein determination. The oxytocin test consists of placing a small portion of fresh mammary tissue in a tube containing 100 mU oxytocin (generously provided by Sandoz) in 0·5 ml saline. If lactogenesis has taken place, the contraction of the mammary tissue produced by the oxytocin will expel the accumulated milk to the surrounding liquid, producing a white opalescence. This is a very sensitive qualitative method for determining lactogenesis (Deis 1968).

In the second experiment, pregnant rats were injected s.c. with 1·5 mg BC/kg or with vehicle at 2000 h on day 20 and at 0800 h on day 21 of pregnancy. They were placed in individual cages and allowed to deliver undisturbed. Those litters with no visible milk in the stomach were adjusted to eight pups and weighed, they were weighed again 12 h later and thereafter every 24 h for the following 7 days. When the litter had decreased in weight in 24 h it was replaced by another litter of approximately

the same age that had been nursed by normal untreated mothers. The same protocol was used with the mothers in which delivery occurred after 0600 h on day 22 or before 2000 h on day 21. Litters born during the night of day 21 showing visible milk in the stomach the next morning were adjusted to eight pups, weighed at 0800 h and every 24 h for the following 7 days. Groups of rats were injected s.c. with four doses of 0·25 mg oPRL, rGH, oGH, pGH, bGH, hGH or rhGH, or 0·5 mg rhGH immediately, 12, 24 and 36 h postpartum. A group of rats was injected with BC and GH vehicle alone.

Casein and lactose determinations

Mammary casein and lactose were measured as previously described (Deis *et al.* 1989). Briefly, 200 mg mammary tissue was cut into small pieces and homogenized in 2 ml 50 mM sodium phosphate buffer, NaCl (150 mM), NaN_3 (0·1%) and Triton X-100 (0·1%) pH 7·6 with an Ultra turrax homogenizer. The homogenate was centrifuged at 600 ***g*** for 30 min and the supernatant used for β-casein determination by a homologous radioimmunoassay according to the method of Edery *et al.* (1984) as modified in our laboratory (Bussmann & Deis 1984). Lactose concentration was assessed by the method of Kuhn & Lowenstein (1967).

Binding assays

Crude membrane prolactin receptor preparations were obtained from livers of rats on day 20 of pregnancy as described by Katoh *et al.* (1987). The livers were rapidly excised after decapitation and stored at −70 °C until membrane preparation. The tissue was homogenized on 5 volumes of ice-cold 3 M sucrose and centrifuged for 15 min at 1500 ***g***. The supernatant was spun for 90 min at 100 000 ***g*** to obtain crude membrane pellets that were resuspended in 2 volumes of Tris–HCl (25 mM) pH 7 and $MgCl_2$ (10 mM) and either used immediately or stored frozen at −30 °C until used. For determination of the affinity of the different GHs for the rat liver membrane preparations, 100 μg liver membrane protein was incubated with ^{125}I-labelled oPRL (50 000–100 000 c.p.m.) in the presence of increasing concentrations (1, 10, 100, 1000 or 10 000 ng/tube) of unlabelled oPRL, rat PRL (rPRL; rat NIDDK-rPRL-B-6 AFP-7545-E), rGH, oGH, hGH, pGH or bGH in a final volume of 0·5 ml Tris–HCl (25 mM), pH 7, $MgCl_2$ (10 mM), bovine serum albumin (0·1%), NaN_3 (0·02%), at room temperature for 16–18 h. The reaction was stopped by dilution with 3 ml reaction buffer and centrifugation. The pellets were counted in a gamma counter. The tracer (NIDDK oPRL-I-2) was labelled with ^{125}I using the chloramine T method and labelled prolactin was separated from free iodine using an acrylamide agarose column (ACA 54; LKB, Bromma, Sweden). Specific activity was 75–80 mCi/mg. The protein content of the membrane preparations was determined by the method of Lowry *et al.* (1951).

Prolactin determination

Groups of control and BC-treated lactating rats were bled by cardiac puncture of the conscious rats at 1800 h on days 1–7 of lactation. Each rat was bled only once, and this procedure did not affect the course of lactation, since the subsequent changes in litter weight were not different from rats that were not bled. Prolactin was measured by double-antibody radioimmunoassay using materials generously provided by the NIADDK (Dr S Raiti, NIADDK Rat Pituitary Hormone Distribution Program, Bethesda, MD, USA). The hormone was radioiodinated using the chloramine T method and purified by passage through Sephadex G-75. The results are expressed in terms of the rat prolactin RP-3 standard preparation. Assay sensitivity was 0·5 μg/l serum and the inter- and intra-assay coefficients of variation were less than 10%.

Statistics

Statistical analysis for the casein and lactose data was performed using one-way or two-way analysis of variance (ANOVA) followed by the least significant difference between means test. The analysis of the daily increase in litter weight was performed using one-way ANOVA followed by the least significant difference between means test, making one ANOVA for each time-point that included all the treatment groups. Student's *t*-test was used to analyse the significance between means for serum prolactin concentration (Snedecor & Cochran 1967). All values are expressed as means ± S.E.M. A level of $P<0·05$ was accepted as statistically significant.

Results

Effect of GH preparations on mammary casein and lactose concentrations in OVX-HYS-BC pregnant rats

Figure 1 shows that administration of 1·5 mg BC/kg immediately after surgery to OVX-HYS pregnant rats abolished the increase in casein and lactose concentration in the mammary glands that was observed 28 h after surgery at 1200 h on day 20. Casein concentrations in the OVX-HYS-BC group were even lower than in untreated rats. Two injections of hGH or rhGH at the 0·5 mg/rat doses or oPRL at 0·25 or 0·5 mg/rat, given immediately after surgery and 12 h later, restored mammary casein concentrations to levels similar to those of the OVX-HYS rats. In contrast, none of the other GHs was able to induce significant increases in casein accumulation, with the exception of oGH at the lower dose which had a slight but significant effect. The

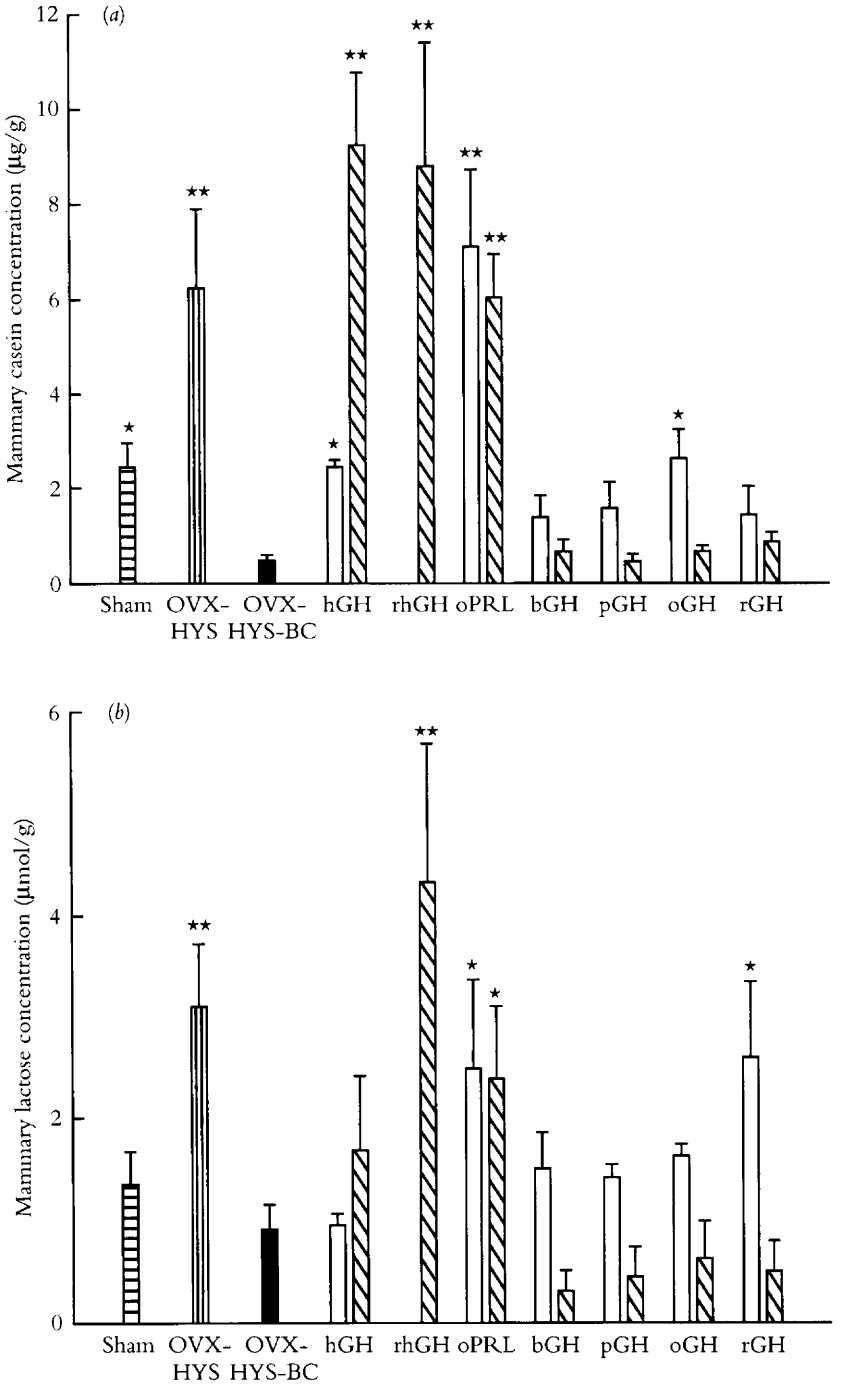

FIGURE 1. (*a*) Casein and (*b*) lactose accumulation in mammary glands from sham-operated (horizontally lined bars, n=7), ovariectomized and hysterectomized (OVX-HYS) rats (vertically lined bars, n=7) or animals treated with bromocriptine (OVX-HYS-BC; solid bars, n=9) on day 20 of pregnancy. Effect of administration of two doses of 0·25 (open bars) or 0·5 (hatched bars) mg/rat of oPRL (n=5 for 0·25 mg dose and n=5 for 0·5 mg), hGH (n=7 and n=5), rGH (n=7 and n=6), oGH (n=6 and n=6), pGH (n=8 and n=6) and bGH (n=8 and n=6) or doses of 0·5 mg rhGH (n=4). Results are means ± S.E.M. ★P<0·05, ★★P<0·01 compared with the OVX-HYS-BC group (ANOVA followed by the least significant difference between means test; Snedecor & Cochran 1967).

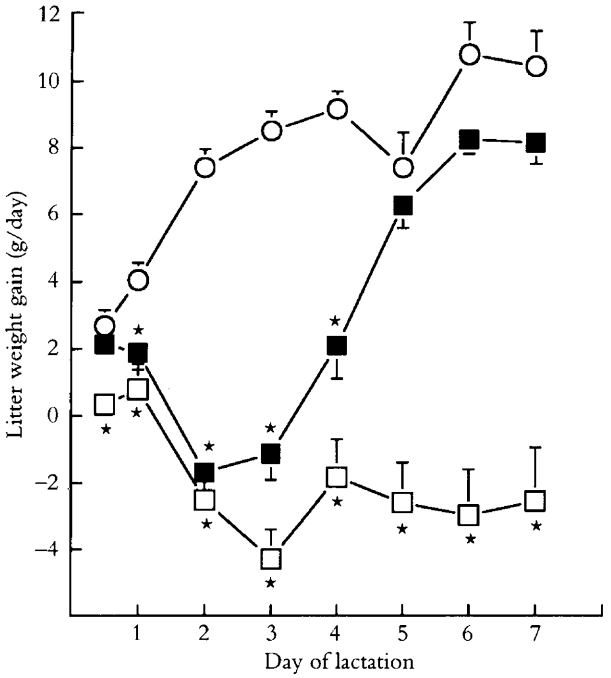

FIGURE 2. Effect of bromocriptine on litter growth in lactating rats. Rats were injected with vehicle (○; n=8) or 1·5 mg bromocriptine/kg (recuperating litters, n=12, ■; non-recuperating litters, n=8, □) at 2000 h on day 20 and at 0800 h on day 21 of pregnancy and the litters were weighed 12 h postpartum and daily from days 1 to 7 of lactation. Values are means ± S.E.M. *P<0·05 compared with controls (one-way ANOVA followed by the least significant difference between means test; Snedecor & Cochran 1967).

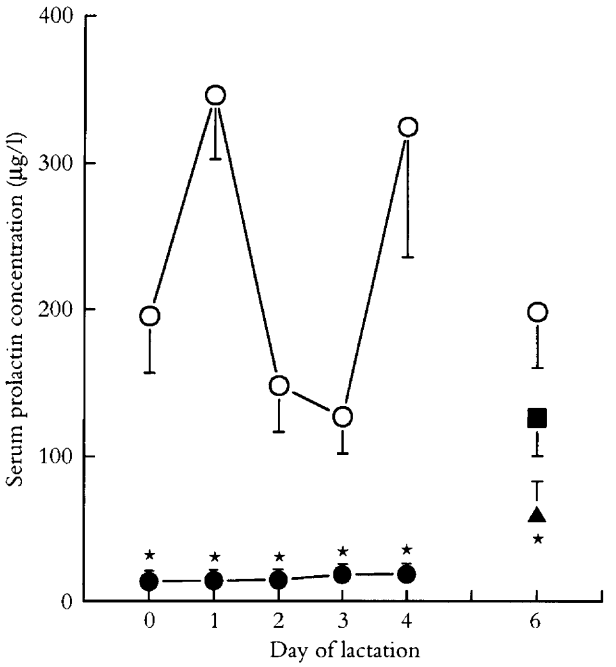

FIGURE 3. Effect of bromocriptine (BC) injections (1·5 mg/kg s.c.) given on days 20 and 21 of gestation on serum prolactin concentration in rats at 1800 h on days 0–6 of lactation. Control rats (○); BC-treated (solid symbols). On day 6 the BC-treated rats could be divided into recuperated (■, n=7) and non-recuperated (▲; n=7) groups. Values represent means ± S.E.M. of groups of seven to ten rats (for the rest of the groups). *P<0·05 compared with control vehicle-treated mothers (Student's t-test).

higher dose of all the other GHs, except hGH or rhGH did not have any effect, giving values similar to the OVX-HYS-BC rats (Fig. 1*a*).

As Fig. 1*b* shows, a significant increase in mammary content of lactose was obtained in the OVX-HYS rats as well as in the groups of OVX-HYS-BC rats receiving 0·25 or 0·5 mg oPRL or 0·5 mg rhGH. In contrast, 0·25 mg hGH did not modify lactose content and only two rats receiving 0·5 mg hGH showed an increase. The 0·25 mg dose of rGH also induced a significant augmentation in mammary lactose concentration, while the low doses of the other GHs produced only non-significant increases. In contrast, lactose content in the rats given the 0·5 mg doses of all the non-human GHs was very low. The oxytocin test demonstrated the presence of milk in the mammary tissues of the OVX-HYS rats and in the OVX-HYS-BC plus oPRL (0·25 or 0·5 mg), or rhGH-treated groups and in two out of five receiving 0·5 mg hGH; these were the same two animals that had an increase in lactose. All the rats given 0·25 mg hGH or 0·25 or 0·5 mg of the non-human GHs had negative oxytocin tests. Our experience with the use of the oxytocin test has shown that a positive response correlates with a simultaneous increase in lactose and casein.

Since the 0·25 mg doses of the non-human GHs gave values of casein and lactose that were always higher than the values obtained after the 0·5 mg dose, we performed a two-way ANOVA comparing the effects of the high and low doses of rGH, oGH, pGH and bGH to ascertain whether there was a differential dose-effect. We found a highly significant dose effect for casein (F=10·37, P<0·005) and lactose (F=23·38, P<0·001), while there was no significant interaction between doses and treatments (F=0·94 and 1·62 respectively).

Effect of BC treatment on litter growth and serum prolactin concentrations during the first 7 days postpartum

BC treatment induced a significant decrease in milk production, as reflected by litter growth, between days 1 and 4 of lactation (Fig. 2). After day 4, litter growth did not recuperate in 33% of BC-treated rats, but the remaining 66% showed increased milk production, reaching control levels on day 5. Interestingly, around 12 h after delivery

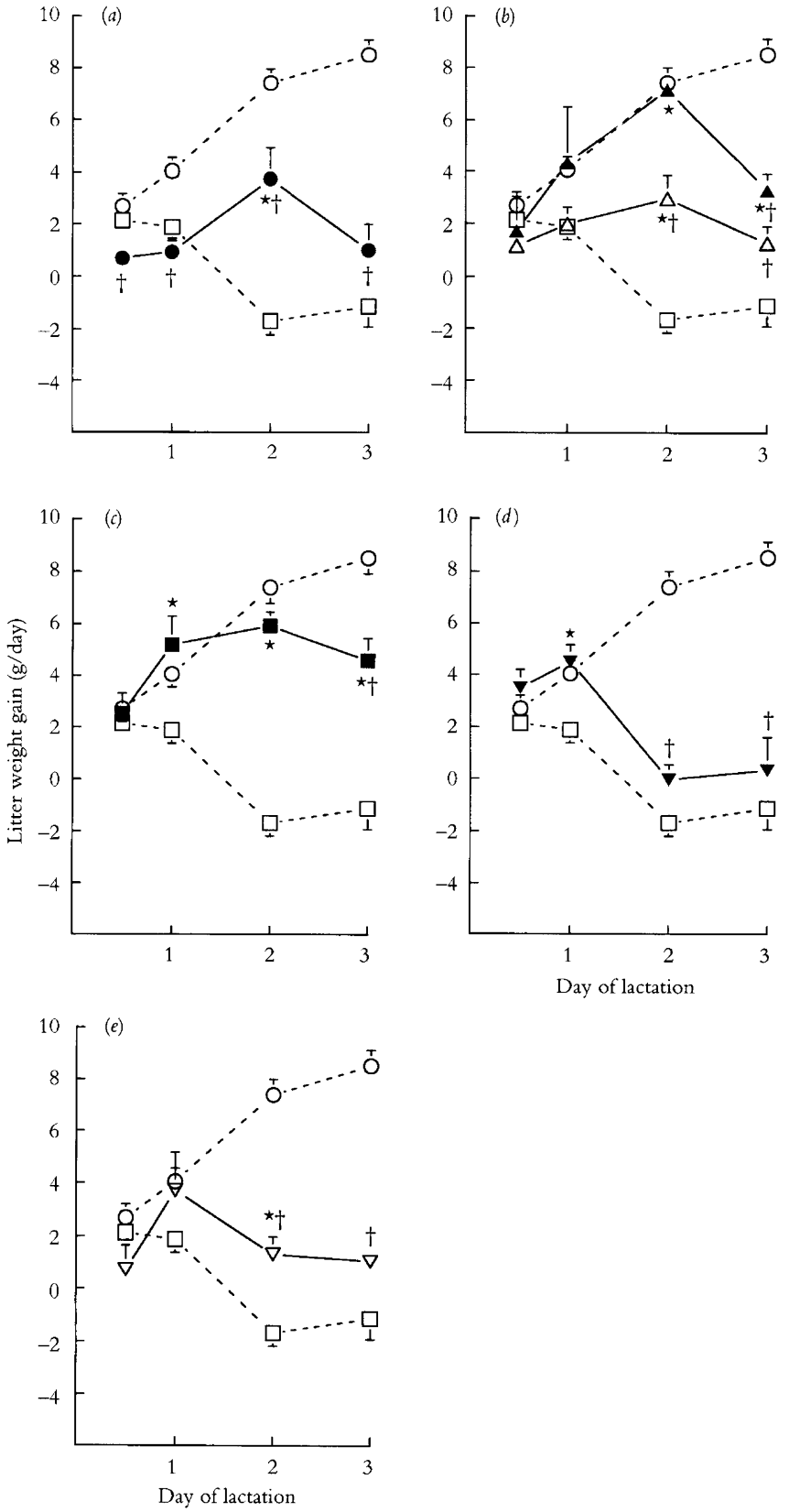
(a)
(b)
(c)
(d)
(e)
Litter weight gain (g/day)
Day of lactation
Day of lactation

TABLE 1. Effect of growth hormones on litter growth in bromocriptine (BC)-treated lactating rats from days 6 to 7 of lactation. Rats were injected with vehicle or BC (1·5 mg/kg) at 2000 h on day 20 and at 0800 h on day 21 of pregnancy and the litters weighed daily. BC treatment induced a significant decrease in litter growth between days 1 and 4 of lactation. After day 4, litter growth did not recuperate in 33% of BC-treated rats (non-recuperating). The remaining 66% showed increased milk production, reaching control levels on day 5 (recuperating). Groups of BC-treated rats were injected with four doses of 0·25 mg (except when indicated otherwise) of oPRL, hGH, rhGH, rGH, oGH, pGH or bGH every 12 h starting immediately postpartum. Results represent the means ± S.E.M. of the increase in weight between days 6 and 7 of lactation of the number of rats in parentheses

	Weight increase of litter (g)	
	Recuperating	Non-recuperating
Controls	10·46 ± 1·03 (8)	—
BC	8·18 ± 0·65 (12)	− 2·53 ± 1·59 (7)*†
+oPRL	4·14 ± 1·53 (8)*†	—
+rhGH (0·25 mg)	3·57 ± 0·81 (6)*†	—
+rhGH (0·50 mg)	9·68 ± 1·57 (4)	—
+hGH	6·12 ± 1·35 (6)*	—
+oGH	5·52 ± 1·42 (6)*	—
+rGH	7·17 ± 1·13 (6)	—
+pGH	8·55 ± 1·10 (4)	− 2·15 ± 3·56 (4)
+bGH	8·25 ± 1·15 (6)	− 3·23 ± 1·44 (3)

*$P<0·05$ compared with controls; †$P<0·05$ compared with recuperating BC-treated group (Student's *t*-test).

there was no difference in litter weight between the control and recuperating BC-treated group. However, in the BC-treated rats which did not recuperate, litter weight 12 h after delivery was significantly lower when compared with both control and recuperating BC-treated mothers (Fig. 2).

Serum prolactin concentrations in the control lactating rats measured at 1800 h on different days postpartum showed values higher than 100 μg/l, while the BC-treated mothers had values always lower than 20 μg/l until day 4. On the afternoon of day 6, values of the BC-treated mothers which had recuperated became similar to those of the control rats, while the serum prolactin concentration in the group which did not recuperate was significantly lower (Fig. 3).

Effect of different GH preparations on litter growth of BC-treated mothers on days 0–3 of lactation

On days 0–2, i.e. during the time of administration and action of the different GH or oPRL treatments (Fig. 4), hGH and the high dose of rhGH restored litter growth to levels similar to the control lactating rats. On day 3 in these two groups, litter growth diminished with respect to controls but was significantly higher than the values of the recuperating BC-treated group (Fig. 4*b* and *c*). Treatments with the low dose of rhGH or with oPRL increased litter growth significantly on day 2 only, while rGH and oGH were effective in significantly elevating milk production only on day 1 or day 2 respectively, compared with the recuperating BC-treated mothers (Fig. 4*a*, *b*, *d* and *e*). In contrast, the pattern of litter growth was not modified by treatment with bGH or pGH, nor was the proportion of rats that did not show recuperation in weight eventually (three out of nine for bGH and four out of eight for pGH, results not shown). The weight change during the first 12 h postpartum in the non-recuperating pGH-treated group was also significantly lower than the gain in the respective recuperating group (0·75 ± 0·71 g compared with 3·85 ± 0·54 g; $P<0·01$), while the difference between the recuperating and non-recuperating groups treated with bGH was not significant (2·75 ± 0·54 g

FIGURE 4. Effect of growth hormones on litter growth in bromocriptine (BC)-treated lactating rats. Rats were injected with vehicle (○; n=8) or 1·5 mg BC/kg (recuperating litters, n=12, □) at 2000 h on day 20 and at 0800 h on day 21 of pregnancy and the litters were weighed 12 h postpartum and daily from days 1 to 3 of lactation. Groups of BC-treated rats were injected with four doses of (*a*) oPRL, 0·25 mg/rat (●, n=9); (*b*) rhGH, 0·25 mg/rat (△, n=5) or 0·5 mg/rat (▲, n=4); (*c*) hGH, 0·25 mg/rat (■, n=5); (*d*) rGH, 0·25 mg/rat (▼, n=6) or (*e*) oGH, 0·25 mg/rat (▽, n=5) every 12 h starting immediately postpartum. Results are means ± S.E.M. *$P<0·05$ compared with the BC-treated recuperating group; †$P<0·05$ compared with controls (one-way ANOVA followed by the least significant difference between means test when more than two experimental groups were compared; Snedecor & Cochran 1967).

compared with 1·13 ± 0·74 g). However, the very small sample of non-recuperating bGH-treated rats may have rendered the difference non-significant.

Litter growth of BC-treated mothers on day 7 of lactation after treatment with different GH preparations

Litters from BC-treated mothers receiving oPRL or the low dose of rhGH showed signs of weight recuperation on day 7 but the values were significantly lower when compared with litters from control intact rats and the recuperating BC-treated mothers, while the high dose of rhGH produced values similar to the control intact rats. Litters from the hGH-treated mothers showed values lower than the control litters, but not different from the recuperating BC-treated group (Table 1). Treatments with oGH or rGH also produced no difference in the recuperating BC-treated mothers and all the rats showed recuperated milk production (Table 1). In contrast, the pGH- or bGH-treated groups showed no significant differences from the BC-treated mothers (Table 1).

Binding of GH preparations to pregnant rat liver lactogenic receptors

Figure 5 shows that the affinity of hGH for liver prolactin receptors was of the same order as oPRL, while that of rhGH was approximately one order of magnitude lower. The capacity of rPRL to displace ^{125}I-labelled oPRL was two orders of magnitude lower. In contrast, all the other GHs had a very low affinity, being able to displace oPRL from the receptor at concentrations that were at least 10 000 times higher.

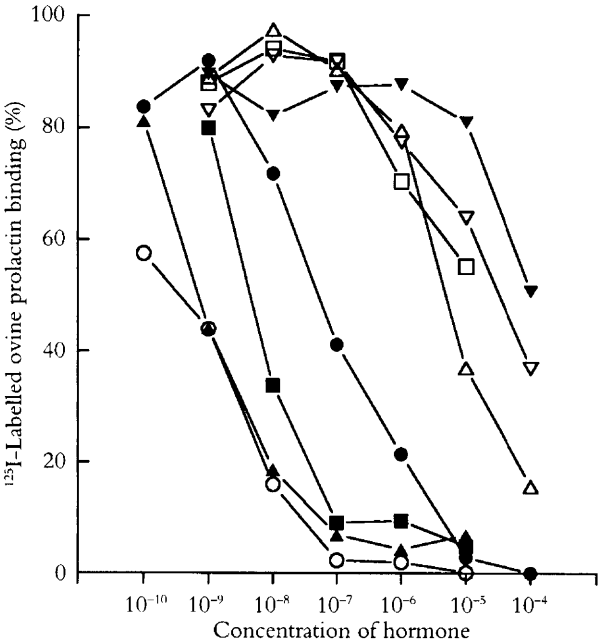

FIGURE 5. Displacement of ^{125}I-labelled oPRL from pregnant rat liver membranes as a function of increasing concentrations of unlabelled oPRL (○), rPRL (●), hGH (▲), rGH (△), oGH (□), bGH (▽), pGH (▼) or rhGH (■) (1 ng/tube to 10 μg/tube). The ordinate represents the percentage of the maximum ^{125}I-labelled oPRL binding, considered as 100%, and which was 30% of total ^{125}I-labelled oPRL added.

Discussion

It is well known that prolactin is the major lactogenic hormone in the rat. Reducing its synthesis and secretion with drugs such as BC decreases but does not abolish milk yield (Flückiger 1978, Cowie *et al.* 1980, Madon *et al.* 1986). The role of GH in lactogenesis and lactation in the rat has not been clearly defined; however, it has been recently shown that GH may have an auxiliary role in lactation, especially in a situation of decreased serum prolactin (Madon *et al.* 1986).

Ovario-hysterectomy performed at the end of pregnancy in rats induced lactogenesis, as shown by the significant increases in mammary casein and lactose concentration as well as by positive oxytocin tests. The induction of lactogenesis was due to a previous increase in prolactin secretion 24 h after surgery (Bussmann *et al.* 1983), and was consequently blocked by the administration of BC, as previously demonstrated by Bussmann *et al.* (1983). Furthermore, the OVX-HYS-BC group had casein values that were significantly lower than those of intact pregnant rats, indicating that prolactin and/or feto-placental secretions may have induced a low rate of casein synthesis in intact pregnant rats.

The well-known ability of hGH to bind and activate lactogenic receptors (Forsyth *et al.* 1965, Kelly *et al.* 1991) can be correlated with the effects of both hGH and rhGH, which induce comparable levels of casein synthesis during lactogenesis at the end of pregnancy. In spite of a similar ability of oPRL and hGH to bind to rat liver prolactin receptor and to induce casein synthesis, hGH was less effective in inducing lactogenesis. Surprisingly, rhGH, having a slightly lower affinity for the receptor, seemed to be as potent as oPRL in inducing lactogenesis. Perhaps slight differences in the ability of hGH or rhGH to activate the prolactin receptor after the binding step are responsible for these differences.

According to our results none of the other GHs used in this study was able to induce full lactogenesis. However, the low dose of oGH induced a small but significant increase in casein, but not in lactose mammary content, and the low dose of rGH induced a significant increase in lactose but not in casein content. Nevertheless, in these two groups the oxytocin test was negative, most probably because only casein or lactose was induced without the

other. The small dose of the other non-human GHs also seemed to have a tendency to increase mammary casein or lactose accumulation. In contrast, the high doses of all the non-human GHs, including oGH and rGH, gave casein and lactose values that were almost equal to those of the OVX-HYS-BC rats. A two-way ANOVA performed to determine whether there was a dose-effect for the non-human GHs gave highly significant results, indicating that the 0·25 mg doses induced higher values of those milk components. At present we have no explanation for the lack of effect of the higher doses. This behaviour was different from that of the groups treated with hGH in which there was a direct dose–response effect, and of those given oPRL in which the maximal response was achieved with both doses.

The results obtained during lactation clearly show that GHs from different species have a varying capacity to stimulate milk production in lactating rats in which lactogenesis had been induced before parturition by placental lactogen in the absence of prolactin. All the BC-treated lactating groups showed an increase in weight during the first 12 h postpartum, demonstrating the presence of available milk during this short period, the synthesis of which had been induced most probably by the remaining action of placental lactogen. In our rats, lactogenesis takes place on the afternoon of day 21 of pregnancy after the fall in progesterone, providing that lactogenic hormones (prolactin or placental lactogen) are present (Deis 1968, Bussmann & Deis 1979, Jahn & Deis 1991). After this initial milk production, BC administration produced a reduction lasting at least until day 4 postpartum, a reduction that was coincident with a significant diminution of prolactin secretion until day 4 of lactation in the recuperating group and at least until day 6 in the non-recuperating mothers. Madon *et al.* (1986) and Flint *et al.* (1992) observed that BC was more effective in decreasing milk production when injected on day 2 of lactation than when the drug was administered on days 6 or 14 postpartum.

In the lactating rats, natural hGH also had the greatest ability to restore milk production. Interestingly, rhGH or oPRL seemed to be less potent than hGH; rhGH mimicked the action of hGH at twice the dose of the latter, and showed the same behaviour as 0·25 mg oPRL. Since rhGH has ten times less affinity for the prolactin receptor from pregnant rat liver than hGH, the lower biological activity of rhGH in this case may be a reflection of its lower affinity for the receptor. We cannot exclude the possibility that, in lactating rats, rhGH may be eliminated from circulation faster than the natural hormone.

Rat and ovine GH had a clear galactopoietic effect on the first day postpartum, showing litter growth similar to the control lactating rats. Thereafter litter growth in both groups was slightly higher than that observed in the recuperating BC-treated group. Most probably this early galactopoietic effect of both hormones allowed all the treated mothers to recover the capacity to produce milk by day 7. Our results confirm and extend those obtained by Madon *et al.* (1986) in which they demonstrate, through the neutralization of endogenous GH with an antibody, that rGH partially stimulates milk production in lactating BC-treated rats on day 4 postpartum. On the other hand, they showed that, in the presence of prolactin, GH antiserum did not modify litter growth, indicating that prolactin action alone can substitute completely for endogenous GH during this stage of lactation (Madon *et al.* 1986). In a more recent work by the same group it was found that rGH antiserum does not modify the effect of BC when given on day 2 of lactation, it potentiates the effect of BC when the treatments were given on day 6 and it can lower milk yield when given alone on day 14 of lactation. These results indicate an increasing role of GH in maintaining lactation as it progresses (Flint *et al.* 1992). Our present results also show a role for GH in the first days of lactation, since rGH and oGH could increase milk production. In contrast, treatment with pGH or bGH seemed to have had no galactopoietic effect whatsoever. We have no explanation for the lack of effect of pGH and bGH when both rGH and oGH were active. Most probably differences in biological activity or affinity for the rat GH receptors may be the reason. The lack of effect of bGH seems to be correlated with the period of lactation when the treatment is initiated. Thus, we could not obtain any effect when it was injected during the first 2 days of lactation in rats treated with BC on days 20 and 21 of pregnancy. However, Madon *et al.* (1986) could reverse the effect of rGH antibody but not of BC administration, with injections of bGH on days 4–6 of lactation to rats pretreated on day 4 with BC plus anti-rGH.

It is worth emphasizing that when a GH had a clear galactopoietic effect on the first 24–48 h postpartum, all the lactating rats treated with BC recovered milk production by day 7, giving growth curves similar to those of the recuperating BC-treated mothers. We may interpret this result as suggesting that there exists a critical period, during the first 24 or 48 h postpartum, for the mammary gland to maintain the capacity to produce milk in response to prolactin, once the effect of BC has ended. Supporting this hypothesis, the rats that did not recover the capacity to re-establish lactation showed a diminished milk production in the first 12 h postpartum.

It is interesting to speculate on the different sensitivity to GH of milk synthesis of the mammary glands of pregnant and lactating rats. This may be due to a different threshold for lactogenic hormones being required for the initiation of milk synthesis at the end of pregnancy, compared with the lactating mammary gland, where milk synthesis has already begun. Conversely, lactogenesis may exclusively require lactogenic hormones. We may hypothesize that the action of non-human GH during pregnancy

could be directed to the stimulation of mammary epithelial growth (Kleinberg *et al.* 1990) while, during lactation, along with this action, GH may optimize milk production, perhaps through a mechanism analogous to that in ruminants.

Acknowledgements

This work was supported by a grant (PID 9036/01) from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. The authors are indebted to Mrs F E Guiñazú de Di Nasso for her excellent technical assistance. We thank Dr A F Parlow and the NIADDK for the gift of the GHs, oPRL and the prolactin radioimmunoassay kit. R P D and G A J are career scientists from the CONICET and R W C has a fellowship from CONICET.

References

Bussmann LE & Deis RP 1979 Studies concerning the hormonal induction of lactogenesis by prostaglandin $F_{2\alpha}$ in pregnant rats. *Journal of Steroid Biochemistry* **11** 1485–1489.

Bussmann LE & Deis RP 1984 Hormonal regulation of casein synthesis at the end of pregnancy. *Molecular and Cellular Endocrinology* **39** 115–118.

Bussmann LE, Koninckx A & Deis RP 1983 Effect of estrogen and placental lactogen on lactogenesis in pregnant rats. *Biology of Reproduction* **29** 535–541.

Carón RW, Jahn GA & Deis RP 1992 Actividad lactogénica y galactopoyética de hormonas de crecimiento de distintas especies en la rata. *X Annual Meeting, Sociedad de Biología de Cuyo*, Mendoza, Argentina. Abstract No 1.

Chene N, de la Llosa P, Charpigny G & Martal J 1986 Relationships between structure and function of lactogenic hormones. *Reproduction Nutrition, Development* **26** 551–561.

Cowie AT 1969 General hormonal factors involved in lactogenesis. In *Lactogenesis*, pp 157–169. Eds M Reynolds & SJ Folley. Philadelphia: University of Pennsylvania Press.

Cowie AT, Forsyth IA & Hart IC 1980 Hormone receptors in mammary gland and the mechanisms of hormone action. In *Hormonal Control of Lactation*, pp 256–258. Eds AT Cowie, IA Forsyth & IC Hart. Berlin: Springer Verlag.

Crowe PD, Buckley AR, Zorn NE & Rui H 1991 Prolactin activates protein kinase C and stimulates growth-related gene expression in rat liver. *Molecular and Cellular Endocrinology* **79** 29–35.

Deis RP 1968 Oxytocin test to demonstrate the initiation and end of lactation in the rat. *Journal of Endocrinology* **40** 133–134.

Deis RP, Carrizo DG & Jahn GA 1989 Suckling-induced prolactin release potentiates mifepristone-induced lactogenesis in pregnant rats. *Journal of Reproduction and Fertility* **87** 147–153.

Deis RP & Jahn GA 1988 Efecto prolongado de bromocriptina (CB-154) sobre la secreción de PRL, progesterona (Pg) y lactancia. Tratamiento con hPL y diferentes hormonas de crecimiento (GH). *XI Meeting of the Asociación Latinoamericana de Investigaciones en Reproducción Humana (ALIRH)*, Puerto Vallarta, México. Abstract No 115.

Edery M, Houdebine LM, Djiane J & Kelly PA 1984 Studies of β-casein content of normal and neoplastic rat mammary tissues by a homologous radioimmunoassay. *Molecular and Cellular Endocrinology* **34** 145–151.

Esquifino AI, Villanua MA & Agrasal C 1987 Possible role of prolactin in growth regulation. *Revista Española de Fisiología* **43** 455–462.

Feldman M, Ruan W, Cunninham BC, Wells JA & Kleinberg DL 1993 Evidence that the growth hormone receptor mediates differentiation and development of the mammary gland. *Endocrinology* **133** 1602–1608.

Flint DJ, Tonner E, Beattie J & Panton D 1992 Investigation of the mechanism of action of growth hormone in stimulating lactation in the rat. *Journal of Endocrinology* **134** 377–383.

Flückiger E 1978 Lactation inhibition by ergot drugs. In *Physiology of Mammary Glands*, pp 71–82. Eds A Yokoyama, H Mizuno & H Nagasawa. Tokyo: Japan Scientific Societies Press and Baltimore: University Park Press.

Forsyth IA 1989 Growth factors in mammary gland function. *Journal of Reproduction and Fertility* **85** 759–770.

Forsyth IA, Folley LSJ & Chadwick A 1965 Lactogenic and pigeon crop-stimulating activities of human pituitary growth hormone preparations. *Journal of Endocrinology* **31** 115–120.

Frankenne F, Closset J, Gomez F, Scippo ML, Smal J & Hennen G 1988 The physiology of growth hormones (GHs) in pregnant women and partial characterisation of the placental GH variant. *Journal of Clinical Endocrinology and Metabolism* **66** 1171–1180.

Houdebine L-M, Djiane J, Dusanter-Fourt I, Martel P, Kelly PA, Devinoy E & Servely J-L 1985 Hormonal action controlling mammary activity. *Journal of Dairy Science* **68** 489–500.

Jahn GA & Deis RP 1991 Involvement of the adrenergic system on the release of prolactin and lactogenesis at the end of pregnancy in the rat. *Journal of Endocrinology* **129** 343–350.

Jammes H, Gaye P, Belair L & Djiane J 1991 Identification and characterisation of GH receptor mRNA in the mammary gland. *Molecular and Cellular Endocrinology* **75** 27–34.

Katoh M, Raguet S, Zachwieja J, Djiane J & Kelly PA 1987 Hepatic prolactin receptors in the rat: characterization using monoclonal antireceptor antibodies. *Endocrinology* **120** 739–749.

Kawauchi H & Yasuda A 1988 The molecular evolution of prolactin and related hormones. In *Prolactin Gene Family and its Receptors*, pp 61–70. Ed K Hoshino. Amsterdam: Excerpta Medica.

Kelly PA, Djiane J, Postel-Vinay MC & Edery M 1991 The prolactin/growth hormone receptor family. *Endocrine Reviews* **12** 235–251.

Kleinberg DL, Ruan W, Catanese V, Newman CB & Feldman M 1990 Non-lactogenic effects of growth hormone on growth and insulin-like growth factor-I messenger ribonucleic acid of rat mammary gland. *Endocrinology* **126** 3274–3276.

Kuhn NJ & Lowenstein JM 1967 Lactogenesis in the rat. Changes in metabolic parameters at parturition. *Biochemical Journal* **105** 995–1002.

Lowry OH, Rosebrough NH, Farr AL & Randall, RJ 1951 Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193** 265–276.

Madon RJ, Ensor DM, Knight CH & Flint DJ 1986 Effects of antiserum to rat growth hormone on lactation in the rat. *Journal of Endocrinology* **111** 117–123.

Ogilvie S, Buhi WC, Olson JA & Shiverick KT 1990 Identification of a novel family of growth hormone-related proteins secreted by rat placenta. *Endocrinology* **126** 3271–3273.

Plaut K, Ikeda M & Vonderhaar BK 1993 Role of growth hormone and insulin-like growth factor-I in mammary development. *Endocrinology* **133** 1843–1848.

Posner BI 1976 Regulation of lactogen specific binding sites in rat liver studies on the role of lactogens and estrogen. *Endocrinology* **99** 1168–1177.

Ruan W, Newman CB & Kleinberg DL 1992 Intact and aminoterminally shortened forms of insulin-like growth factor-I

induce mammary gland differentiation and development. *Proceedings of the National Academy of Sciences of the USA* **89** 10872–10876.
Snedecor GW & Cochran WG 1967, *Statistical Methods*. Ames, Iowa: Iowa State University Press.
Tiong TS & Herington AC 1991 Tissue distribution, characterization, and regulation of messenger ribonucleic acid for growth hormone receptor and serum binding protein in the rat. *Endocrinology* **129** 1628–1634.
Tucker HA 1988 Lactation and its hormonal control. In *Physiology of Reproduction*, vol 2, pp 2235–2263. Eds E Knobil & JD Neill. New York: Raven Press.

Received 26 January 1993
Final revision received 21 December 1993
Accepted 21 April 1994