Effect of prolactin and growth hormone on prolactin and LH receptors in the dwarf mouse

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Summary. Dwarf mice (DW/J; *dw/dw*) which exhibit a deficiency of prolactin and GH secretion were treated for 8 days with ovine prolactin and/or human GH (10 or 20 µg/day) and the effect on hepatic and testicular prolactin receptors was investigated. In both sexes there was a significant increase in body weight after all hormone treatments, but an increment in testicular weight was observed only after prolactin administration. Prolactin treatment increased the specific binding % of prolactin in liver membranes in females but not males, and in testicular homogenates (together with an increase in LH receptors). The results suggest that lack of prolactin but not of GH retards sexual development in these mice. Treatment with prolactin partly counteracts this deficiency, and the effect may be mediated by the induction of hepatic and testicular prolactin and LH receptors.

Introduction

The mutant dwarf mouse inbred DW/J strain (mutant gene *dw/dw*) first reported by Snell (1929) is deficient in pituitary prolactin (Bartke, 1964, 1965a; Dung, 1975), whereas growth hormone (GH) is present in trace amounts in homogenates of pituitaries (Dung, 1975). A more recent report by Sinha, Salocks & Vanderlaan (1975), who used homologous radioimmunoassays, confirmed the lack of GH, particularly in males, as well as the profound deficiency of prolactin. Pituitary gonadotrophin concentrations, however, appear normal (for references see Lewis, 1967). Transplantation of normal pituitary glands to the kidney capsule of dwarf mice results in chronic hypersecretion of prolactin (Bartke, 1965b) and will restore fertility in male (Bartke, 1966a) and female (Bartke, 1966b) dwarf mice. A similar effect was observed after the administration of ovine prolactin.

In a previous report we have shown that the increase in binding sites for prolactin in the liver of hypophysectomized rats requires the presence of GH or ACTH as well as prolactin (Bohnet, Aragona & Friesen, 1976; Aragona, Bohnet, Fang & Friesen, 1976). Since the LH receptor in male (Bohnet, Aragona & Friesen, 1975; Aragona, Bohnet & Friesen, 1976) and female (Holt, Richardson, Midgley & Reichert, 1976) gonads is known to be affected by prolactin, the present study was undertaken to examine whether the increase in prolactin binding sites in the liver and prolactin and LH binding sites in the gonad occurs after treatment with prolactin alone or whether GH is needed to potentiate the effect.

Materials and Methods

Homozygous dwarf mice (*dw/dw*) of the strain DW/J and phenotypically normal mice, some of which might have been heterozygous (*dw/+*), were purchased from the Jackson Laboratory, Bar Harbor, Maine, when 50 days of age. Animals were maintained in standard conditions for at least 1 week after arrival before experiments were started.

Ovine prolactin (NIH-P-S12, 35 i.u./mg; GH activity was 0.01 i.u./mg by bioassay) and/or human GH (1.15 i.u./mg; contamination with human prolactin was 0.16% by specific radioimmunoassay) were injected s.c. daily for 8 days as a dose of 10 or 20 µg. The hormones were dissolved

in a minimal amount of 0.1 M-NH₄HCO₃ and further diluted with 0.9% (w/v) NaCl to a final concentration of 100 µg/ml. Controls were injected with the same amount of vehicle. Animals were weighed daily, bled by incision of the tail veins and killed by cervical dislocation. The liver and testes were removed, dissected free of adherent tissue and weighed.

Liver membranes (100,000 g pellet) were prepared and assayed for prolactin and GH binding using ¹²⁵I-labelled human GH as tracer and bovine GH for displacement as described previously (Posner, Kelly, Shiu & Friesen, 1974). Testes from 2 or 3 dwarf mice were pooled. Tissues were homogenized in a glass homogenizer (Pyrex) with tris-HCl buffer containing 25 mM-MgCl₂ using a ratio of 200 mg tissue/ml buffer. Protein content was measured by the procedure described by Lowry, Rosebrough, Farr & Randall (1951). Binding for prolactin and LH was determined using 500 µg testicular homogenate protein/tube and 100,000 ct/min of ¹²⁵I-labelled ovine prolactin or ¹²⁵I-labelled bovine LH as tracer (for details see Aragona *et al.*, 1976).

Serum GH (3 determinations of sera pooled from 3 dwarf mice) was measured by radioimmunoassay using the kit for rat GH supplied by NIH and was expressed as ng of the NIH mouse GH standard, AFP-689-B/ml.

Results

The mean $\pm$ S.E.M. body weight of normal male mice was 25.1 $\pm$ 2.3 g (N = 10) which was slightly greater than that of females (22.2 $\pm$ 0.6 g; N = 10). There were no significant sex differences in body weights of the dwarf mice which ranged between 5 and 10.5 g with an average of 7.7 $\pm$ 0.2 g (N = 53). The percentage increases of body weight after treatment are shown in Table 1. A dose of 10 µg ovine prolactin/day appeared to be more effective in the female dwarf mice, but all treatments had similar effects in the males. Treatment with either dose of ovine prolactin doubled the weight of the testes of dwarf mice but the weight was less than that of normal males (Table 2).

Table 1. Mean $\pm$ S.E.M. percentage increases of and actual (in parentheses, g) body weight of female and male dwarf mice treated with ovine prolactin and/or human GH

Treatment (dose/day)	Females			Males		
	No. of mice	Days of treatment		No. of mice	Days of treatment	
		0	8		0	8
Vehicle (normal mice)	5	100.0 $\pm$ 0.7 (22.8 $\pm$ 1.4)	97.4 $\pm$ 3.7	4	100.0 $\pm$ 3.1 (27.5 $\pm$ 0.87)	100.9 $\pm$ 2.3
Vehicle (dwarf mice)	3	100.0 $\pm$ 1.8 (7.6 $\pm$ 0.14)	99.9 $\pm$ 1.2	3	100.0 $\pm$ 5.7 (7.9 $\pm$ 0.45)	100.0 $\pm$ 5.6
Ovine prolactin, 10 µg	3	100.0 $\pm$ 3.2 (10.1 $\pm$ 0.33)	159.7 $\pm$ 12.0	6	100.0 $\pm$ 12.6 (6.0 $\pm$ 0.76)	136.7 $\pm$ 22.3*
Ovine prolactin, 20 µg	9	100.0 $\pm$ 3.7 (7.06 $\pm$ 0.23)	127.1 $\pm$ 5.4	9	100.0 $\pm$ 4.7 (7.4 $\pm$ 0.46)	116.5 $\pm$ 6.7
Human GH, 10 µg	4	100.0 $\pm$ 4.5 (7.3 $\pm$ 0.33)	118.2 $\pm$ 2.5	—	—	—
Human GH, 20 µg	3	100.0 $\pm$ 3.7 (7.2 $\pm$ 0.23)	125.0 $\pm$ 5.4	4	100.0 $\pm$ 3.7 (7.2 $\pm$ 0.27)	125.0 $\pm$ 5.4
Ovine prolactin, 10 µg +human GH, 10 µg	3	100.0 $\pm$ 10.8 (8.3 $\pm$ 0.9)	137.0 $\pm$ 7.9*	4	100.0 $\pm$ 3.5 (7.1 $\pm$ 0.12)	124.6 $\pm$ 2.7

* Significant weight gain on Day 8 after treatment, $P < 0.05$; all other groups treated with prolactin and/or GH were significantly different from controls, $P < 0.01$.

In untreated male and female dwarf mice specific binding sites for prolactin were virtually undetectable in liver membranes. Treatment with ovine prolactin or human GH alone did not increase the specific binding sites for prolactin in the liver of the male, but there was a significant increase in the females except when GH was given alone (Table 2).

Table 2. Mean $\pm$ S.E.M. specific binding (%) of prolactin in liver membranes of dwarf mice after the administration of ovine prolactin and/or human GH (no. of animals in parentheses)

Treatment (dose/day)	Prolactin binding		GH binding	
	Male	Female	Male	Female
Vehicle (normal mice)	7.4 $\pm$ 0.6* (4)	16.8 $\pm$ 0.6* (7)	1.6 $\pm$ 0.3 (3)	2.9 $\pm$ 0.2* (5)
Vehicle (dwarf mice)	0.2 $\pm$ 0.2 (4)	0.3 $\pm$ 0.3 (4)	1.9 $\pm$ 0.2 (4)	1.7 $\pm$ 0.4 (4)
Ovine prolactin, 10 μ g	0.6 $\pm$ 0.6 (3)	7.6 $\pm$ 0.9* (3)	—	—
Ovine prolactin, 20 μ g	0 (3)	5.5 $\pm$ 0.9* (3)	—	—
Ovine prolactin, 10 μ g + human GH, 10 μ g	0 (3)	9.8 $\pm$ 2.0* (3)	—	—
Human GH, 20 μ g	0 (3)	0.7 $\pm$ 0.4 (3)	—	—

* Value significantly different from that of control dwarf mice, $P < 0.01$.

The specific binding for GH in liver membranes of male dwarf mice ($1.9 \pm 0.2\%$, $N = 4$) and normal males ($1.6 \pm 0.3\%$, $N = 3$) was similar, whereas in normal females the value was $2.9 \pm 0.2\%$ ($N = 5$) and in mutants was $1.7 \pm 0.4\%$ ($N = 4$) ($P < 0.01$).

The effects of treatment on specific binding in testicular homogenates of dwarf mice are shown in Table 3.

Table 3. Mean $\pm$ S.E.M. testicular weight (mg) and specific binding (%) of prolactin and LH to a homogenate of testicular tissue from dwarf mice after treatment with ovine prolactin and/or human GH

Treatment (dose/day)	No. of mice	Testicular wt (mg)	Prolactin	LH
Vehicle (normal mice)	8	304.5 $\pm$ 8.5*	1.7 $\pm$ 0.2	2.5 $\pm$ 0.2
Vehicle (dwarf mice)	9	65.9 $\pm$ 3.3	0.2 $\pm$ 0.1	1.2 $\pm$ 0.1
Ovine prolactin, 10 μ g	3	120.5 $\pm$ 3.1*	0.6 $\pm$ 0.2*	1.9 $\pm$ 0.1**
Ovine prolactin, 20 μ g	8	128.9 $\pm$ 9.9*	1.0 $\pm$ 0.1*	1.8 $\pm$ 0.1**
Ovine prolactin, 10 μ g + human GH, 10 μ g	3	123.7 $\pm$ 2.7*	0.6 $\pm$ 0.2*	1.9 $\pm$ 0.2**
Human GH, 20 μ g	3	68.9 $\pm$ 3.7	0.3 $\pm$ 0.1	1.3 $\pm$ 0.2

Significantly different from the control dwarf mice, * $P < 0.05$, ** $P < 0.01$.

The mean $\pm$ S.E.M. serum GH concentration in normal females (23.2 ± 0.9 ng/ml; $N = 3$) was higher than that in males (17 ± 0.6 ng/ml, $N = 3$). The GH level in male dwarf mice (18 ± 0.6 ng/ml, $N = 3$) was similar to that observed in female dwarfs (18.3 ± 0.9 ng/ml, $N = 3$), but the latter value was significantly lower ($P < 0.01$) than that of normal females.

Discussion

These results indicate that treatment of mutant dwarf mice with prolactin at a dose of 30 to 100 times less than that used in other studies (Bartke, 1966a,b) stimulates growth. The growth-promoting effect of prolactin is perhaps not entirely unexpected, because antiserum to prolactin inhibited subsequent growth of the animals when given to newborn mice (Sinha, Lewis & Vanderlaan, 1971). Moreover, when rat livers were perfused *in situ*, it was shown that the addition of 50 ng ovine prolactin/ml significantly stimulated somatomedin production, whereas 1 μ g bovine GH was required

to elicit a similar response (Francis & Hill, 1975). The effect of prolactin in the present experiments might be mediated by the induction of hepatic and gonadal lactogenic receptors, but the precise site of action remains unknown. Several pituitary hormones are required to maintain prolactin receptors in hypophysectomized rats and a combination of prolactin, GH and ACTH was most effective (Bohnet *et al.*, 1976; Aragona *et al.*, 1976). Prolactin treatment increased the number of LH receptors in the dwarf mouse testis as it does in rat testis (Bohnet *et al.*, 1975; Aragona *et al.*, 1976), but human GH alone had no effect on prolactin binding sites in liver and testis or on testicular growth. This is consistent with the findings that prolactin, but GH only exceptionally, was able to induce fertility in dwarf mice (Bartke, 1963, 1964).

Prolactin seems to have a stimulatory effect on testosterone secretion in rodents (Hafiez, Lloyd & Bartke, 1972; Bartke, 1973), while testosterone decreases hepatic prolactin receptors in the rat liver (Posner, Kelly & Friesen, 1974). Whether such a mechanism or an unbalanced hormonal situation could explain the failure to induce prolactin receptors in the liver of the male dwarf mouse remains unknown, as in normal males such receptors were readily detected.

Dung (1975) has shown that prolactin cells constituted 10% of the entire cell population in the pituitary gland of dwarf mice, whereas in control mice the proportion was >40%. The percentages of GH cells in the two groups were similar and this observation is consistent with the fact that the dwarf mouse is not totally deficient in GH (Sinha *et al.*, 1975).

Our present results suggest that the lack of normal growth and fertility in the dwarf mutant mouse is probably not due to a lack of GH and its receptor but to greatly diminished pituitary and serum prolactin concentrations and to a lack of reasonable numbers of hepatic and gonadal prolactin receptors.

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References

- ARAGONA, C., BOHNET, H.G., FANG, V.S. & FRIESEN, H.G. (1976) Induction of lactogenic receptors. II. Studies on the liver of hypophysectomized male rats and on rats bearing a growth hormone secreting tumor. *Endocr. Res. Commun.* **3**, (in press).
- ARAGONA, C., BOHNET, H.G. & FRIESEN, H.G. (1976) Localization of prolactin binding in prostate and testis: the role of serum prolactin concentration on the testicular LH receptor. *Acta endocr., Copenh.* (in press).
- BARTKE, A. (1963) The response of two genetically different types of dwarf mice to growth hormone. *Genetics, Princeton* **48**, 882.
- BARTKE, A. (1964) Histology of the anterior hypophysis, thyroid and gonads of two types of dwarf mice. *Anat. Rec.* **149**, 225-236.
- BARTKE, A. (1965a) Influence of luteotrophin on fertility of dwarf mice. *J. Reprod. Fert.* **10**, 93-103.
- BARTKE, A. (1965b) Absence of prolactin activity in pituitary of dwarf mice. *Genetics, Princeton* **52**, 426-427.
- BARTKE, A. (1966a) Influence of prolactin on male fertility in dwarf mice. *J. Endocr.* **35**, 419-420.
- BARTKE, A. (1966b) Reproduction of female dwarf mice treated with prolactin. *J. Reprod. Fert.* **11**, 203-206.
- BARTKE, A. (1973) Plasma testosterone levels in male mice and rats treated with inhibitors of prolactin release. *Acta endocr., Copenh., Suppl.* **177**, 22, Abstr.
- BOHNET, H.G., ARAGONA, C. & FRIESEN, H.G. (1975) Effect of changes in serum prolactin on prolactin and gonadotropin binding to Leydig cells and tubules of the rat testis. *Clin. Res.* **23**, 613A, Abstr.
- BOHNET, H.G., ARAGONA, C. & FRIESEN, H.G. (1976) Induction of lactogen receptors. I. In the liver of hypophysectomized female rats. *Endocr. Res. Commun.* **3**, (in press).
- DUNG, H.G. (1975) Evidence of prolactin cell deficiency in connection with low reproductive efficiency of female 'torpid' mice. *J. Reprod. Fert.* **45**, 91-99.
- FRANCIS, M.J.O. & HILL, D.J. (1975) Prolactin-stimulated production of somatomedin by rat liver. *Nature, Lond.* **255**, 167-168.
- HAFIEZ, A.A., LLOYD, C.E. & BARTKE, A. (1972) The role of prolactin in the regulation of testis function: the effects of prolactin and luteinizing hormone on the plasma levels of testosterone and androstenedione in hypophysectomized rats. *J. Endocr.* **52**, 327-332.
- HOLT, J.A., RICHARDSON, J.A.S., MIDGLEY, A.R. & REICHERT, L.E. (1976) Effect of prolactin on LH receptors in rat luteal cells. *Endocrinology* **98**, 1005-1013.
- LEWIS, U.J. (1967) Growth hormone of normal and dwarf mice. *Mem. Soc. Endocr.* **15**, 179-188.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. &

- RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265-275.
- POSNER, B.I., KELLY, P.A., SHIU, R.P.C. & FRIESEN, H.G. (1974) Studies of insulin, growth hormone and prolactin binding: tissue distribution, species variation and characterization. *Endocrinology* **95**, 521-531.
- POSNER, B.I., KELLY, P.A. & FRIESEN, H.G. (1974) Induction of lactogenic receptor in rat liver: influence of estrogen and the pituitary. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2407-2410.
- SINHA, Y.N., LEWIS, U.J. & VANDERLAAN, W.P. (1971) Effects of prolactin and growth hormone deficiency on the growth of neonatal mice. *Proc. 53rd Meeting, Endocr. Soc.*, A-125, Abstr.
- SINHA, Y.N., SALCOCKS, C.B. & VANDERLAAN, W.P. (1975) Pituitary and serum concentrations of prolactin and GH in Snell dwarf mice. *Proc. Soc. exp. Biol. Med.* **150**, 207-210.
- SNELL, G.D. (1929) Dwarf, a new mendelian recessive character of the house mouse. *Proc. natn. Acad. Sci. U.S.A.* **15**, 733-734.

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