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Prolactin similar to ectopic pituitary isograft restores responsiveness in Snell dwarf mice

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THE effects of ovine prolactin (PRL) (2 × 5 IU a day) and an ectopic pituitary isograft on the responsiveness were examined using locomotor and exploratory activities as measures in PRL-growth-hormone-thyrotropin-deficient Snell dwarf mice (*dw/dw*). After 5 weeks of treatment, both PRL and the graft restored the two behavioural measures to normal levels. Results clearly demonstrate the involvement of PRL in global behavioural responsiveness and suggest a possible role for PRL in the changes induced by the graft.

Key words: Snell dwarf mouse (*dw/dw*); Dwarfism; Responsiveness; Locomotion; Exploration; Behavioural improvement; Prolactin; Pituitary graft

Introduction

In producing prolactin (PRL) in physiologically significant amounts,[1] pituitaries transplanted into an ectopic site have constituted a model of induced hyperprolactinemia, widely used in studies of the involvement of PRL in physiological[1] and, to a lesser extent, behavioural processes in rodents. In this respect, it has been reported that in rats such hyperprolactinemia induces hypergrooming,[2] facilities maternal behaviour[3] and acquisition of avoidance behaviours, diminishes responsiveness to electrical footshock,[4] and disturbs male sexual behaviour.[5] Furthermore, we have shown that ectopic pituitary graft restores to normal levels spontaneous alternation, passive avoidance,[6] exploratory[7] and neotic[8] behaviours of the homozygous Snell dwarf mouse,[9] characterized by a defective anterior pituitary gland[10] secreting low amounts of growth hormone (GH), PRL[11] and thyrotropin (TSH).[12] However, it has recently been demonstrated in rats that such an ectopic pituitary graft could likewise secrete GH.[13] The present study was designed to examine the respective effects of PRL and ectopic pituitary isograft treatments on responsiveness as measured by locomotor and exploratory activities in dwarf mice.

Materials and Methods

Subjects: Snell dwarf mice of the inbred strain DW/Orl-dw, originally obtained from the Centre de Sélection et d'Elevage d'Animaux de Laboratoire (CNRS, Orléans La Source, France) were produced in our breeding colony by mating phenotypically normal female carriers of the *dw* gene with homozygous deficient male dwarfs bearing two ectopic pituitary isografts from adult control females.[7] At weaning (21 days), four groups of ten male mice were formed, consisting of grafted dwarfs plus saline (graft *dw*), sham-grafted dwarfs plus PRL (sham *dw* + PRL), sham-grafted dwarfs plus saline (sham *dw*) and sham-grafted heterozygous controls plus saline (sham c).

Environment: Because of aggressive behaviour by controls toward dwarf mice, immediately after surgery the animals were housed in pairs according to phenotype and treatment. They were maintained from birth in a room with a controlled 12 h light/dark cycle with lights on at 0800 h and temperature maintained at 23°C. Standard rodent pellets and water were available *ad libitum*.

Anaesthesia: The operations were performed at weaning under i.p. anaesthesia (6% pentobarbital: 0.9 $\mu l\ g^{-1}$ body weight + 0.9% NaCl: 5.6 $\mu l\ g^{-1}$ body weight + atropine sulfate, 0.4mg ml^{-1}: 3.4 $\mu l\ g^{-1}$ body weight).

Surgery: Grafted animals received a single pituitary from a male adult control of the same strain, half under each kidney capsule. Sham-grafted animals underwent the same surgical intervention without receiving any transplant.

Hormone treatment: Ovine PRL (oPRL) obtained from Sigma (25 IU mg^{-1}) was dissolved in sterile 0.9% NaCl, pH 7.4, then stored at −20°C. From the day after surgery, mice were injected subcutaneously in the neck with either PRL (5 IU in 0.1 ml of saline) or saline (0.1 ml) twice daily (0900 h and 1900 h), according to treatment. On the day of the test, however, animals received the daily dose in a single injection after testing. The dose and protocol of injections used were based on studies by Bartke[14] and Bartke and Lloyd[15] showing

respectively that 5 IU PRL given twice daily represented the minimal treatment necessary to induce pseudopregnancy in adult control mice, and that both 10 IU PRL daily for 28 days and a single ectopic pituitary isograft resulted in marked stimulation of spermatogenesis in dwarfs.

Procedure: A significant increase in body weight was taken to indicate that the graft has taken successfully.[6,7] For this reason animals were weighed weekly for 8 weeks. Behavioural measures were made after 5 weeks of treatment in a polyvinyl chloride box (30 × 20 × 20 cm) subdivided into 6 equal square exploratory units, all communicating by small doors, and covered with transparent plexiglass. The box could be divided into half lengthwise by 3 movable doors so that the middle openings could be opened or closed. During observations, the experimenters always stood beside the box at the same place. Twenty-four hours before testing, each mouse was placed in one half of the apparatus with the temporary partitions in place and was thus familiarized with one side of the apparatus. The animal was given unlimited access to food and water. The next day, each subject was simultaneously given access to the familiar and novel environments by removing the partitions. This procedure did not involve removing the subject from the box. The mouse was then observed for 10 min in red light, between 1400 h and 1800 h. To take the size difference between the animals of the 4 groups into account, we expressed locomotor activity by the number of steps in each compartment. This variable was computed by multiplying for each compartment the total number of units entered over the 10 min test by a constant (2.008, 2.8, 3.26 and 3.26 respectively for sham c, graft *dw*, sham *dw* + PRL and sham *dw*). These constants were computed in a separate experiment in which we measured the distance covered and the steps taken during a 5 s period by animals of each treatment group. Furthermore, exploratory activity was estimated by the number of rearings in the apparatus.

Statistical analysis: Data were analyzed using Levene's test for equal variances. One-way ANOVA (with replication when necessary) was performed (using Brown-Forsythe's test for unequal variances) followed by Bonferroni's *t*-test[16] or multiple comparisons (with separate variance *t* for unequal variances and pooled variances *t* for equal variances).

Results

Morphological measures: The body weight and body weight increase of mice for each of the 8 weeks of treatment is shown in figure 1. The graft induced a body weight increase beginning from the first week of treatment ($F_{3,19} = 463.4$, $p < 0.001$; $t_{14} = 5.57$, $p < 0.001$ for the comparison with sham *dw*). The PRL treatment induced a similar effect from the second week. ($F_{3,20} = 880.91$, $p < 0.001$; $t_{13} = 6.01$, $p < 0.001$). However the effect of PRL was weaker than that of the graft over the entire treatment period ($p < 0.001$ in all cases) and was less durable, since it was significant only from the 2nd to the 5th week of treatment ($p < 0.01$), compared to the treatment period for the graft ($p < 0.001$ in all cases). Furthermore, graft *dw* never reached the weight of controls ($p < 0.001$).

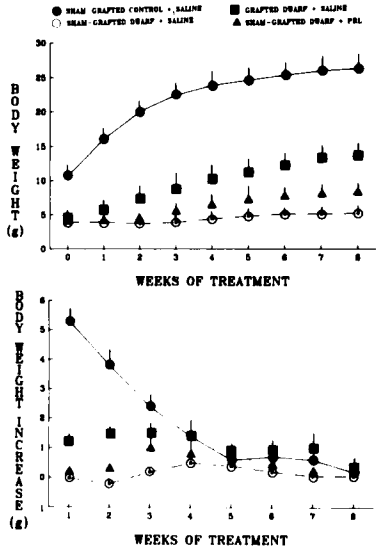

FIG. 1. Body weight and body weight increase (mean ± s.d.) in sham c (●), graft *dw* (■), sham *dw* + PRL (▲) and sham *dw* (○) over the 8 weeks of treatment.

Behavioural measures: With regard to locomotion (Fig. 2) in the familiar compartment, sham *dw* showed

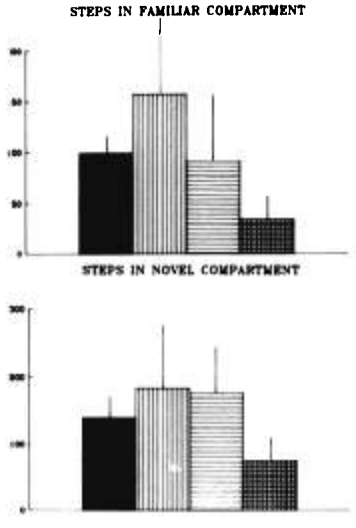

FIG. 2. Locomotor activity measured by the number of steps (mean ± s.d.) in familiar and novel compartments in sham c (▓), graft *dw* (||||), sham *dw* + PRL (≡) and sham *dw* (###) over the 10 min of

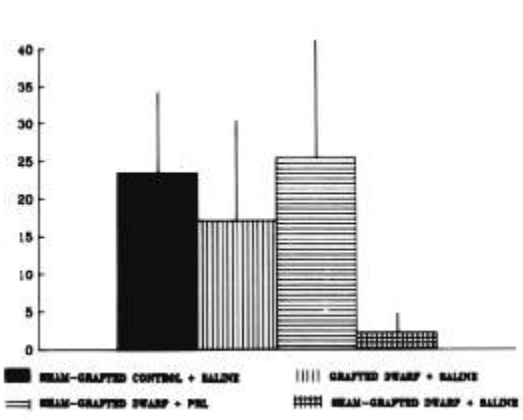

FIG. 3. Exploratory activity measured by the number of rearing activities (mean ± s.d.) in sham c (■), graft *dw* (III), sham *dw* + PRL (≡) and sham *dw* (###) over the 10 min of test.

fewer steps than did the three other groups of mice ($F_{3,21} = 8.97$, $p < 0.001$; $t_{16} = 6.59$, $p < 0.001$; $t_{10} = 4.89$, $p < 0.01$ and $t_{14} = 3.21$, $p < 0.05$ respectively for the comparisons with sham c, graft *dw* and sham *dw* + PRL), which did not differ from each other. Similar results were obtained for locomotion in the novel compartment ($F_{3,22} = 6.14$, $p < 0.01$; $t_{17} = 4.14$, $p < 0.01$; $t_{11} = 3.42$, $p < 0.05$ and $t_{13} = 4.08$, $p < 0.01$).

Results for exploratory activity are presented in figure 3. Sham *dw* reared less frequently than did sham c ($F_{3,25} = 7.53$, $p < 0.001$; $t_9 = 5.88$, $p < 0.001$), graft *dw* ($t_9 = 3.31$, $p < 0.05$) and sham *dw* + PRL ($t_9 = 4.48$, $p < 0.01$); the last three did not differ from each other.

Discussion

The results of the present experiment confirm the growth-promoting effect of PRL[15,17] and ectopic pituitary graft[6,7,15] in *dw* mice.

We did not consider it necessary to assess the biological activity of oPRL, being aware of the possibility of formation of antibodies to the heterologous PRL preparation.[18] Furthermore, a recent study has demonstrated that the effect of PRL on copulatory behaviour in rats was more dependent upon time of exposure to the treatment than upon dose.[5]

In behavioural terms, the present study shows in *dw* mice that after 5 weeks of treatment, oPRL, like the ectopic pituitary graft, restores both locomotor activity and exploratory activity as measured here to normal levels. In other words, oPRL and the graft induce similar behavioural changes, clearly demonstrating the involvement of PRL in global behavioural reponsiveness in dwarf mice. Results suggest a possible role for PRL in the changes observed after the graft. The present results are inconsistent with those obtained by Drago *et al*[19] showing no effect of grafts on locomotor and rearing behaviours in normal rats. Although PRL also reaches elevated levels in grafted *dw* mice,[20] this divergence of results could be explained by the physiological state of the target organs.[21]

It has been demonstrated that PRL could modulate dopaminergic transmission in several brain areas not related to endocrine control, such as the nigro-striatal pathway.[22] In this regard, a large reduction in striatal dopaminergic activity and release has been shown in dwarf mice.[23] Furthermore, it has been suggested that the behavioural effects of PRL on several kinds of unconditioned[2] as well as conditioned behaviours[4] might be due to enhanced dopaminergic transmission in certain brain areas, including the nigro-striatal system. Thus, there are good grounds for believing that increased dopaminergic activity induced by PRL could account for the enhanced responsiveness exhibited both by oPRL and grafted treated mutants.

Conclusion

The present results demonstrate the involvement of PRL in global behavioural responsiveness in Snell dwarf mice and suggest a possible role for PRL in the recovery of reactivity observed after ectopic pituitary graft.

References

1. Adler RA. *Endocrine Rev* **7**, 302–313 (1986).
2. Drago F, Bohus B. *Behav Neural Biol* **33**, 117–122 (1981).
3. Bridges RS, Dunckel PT. *Biol Reprod* **37**, 518–526 (1987).
4. Drago F, Bohus B, Mattheij JAM. *Physiol Behav* **28**, 1–4 (1982).
5. Doherty PC, Bartke A, Smith MS *et al. Horm Behav* **19**, 111–121 (1985).
6. Bouchon R, Ropartz Ph. *J Physiol* **79**, 129–131 (1984).
7. Bouchon R, Ropartz Ph. *Physiol Behav* **36**, 597–602 (1986).
8. Bouchon R, Misslin R, Vogel E *et al. Behav Proc* **15**, 155–165 (1987).
9. Snell GD. *Proc Natl Acad Sci* **15**, 733–734 (1929).
10. Carsner RL, Rennels EG. *Science* **131**, 829 (1960).
11. Slabaugh MB, Lieberman ME, Rutledge JJ *et al. Endocrinology* **109**, 1040–1046 (1981).
12. Roux M, Bartke A, Dumont F *et al. Cell Tissue Res* **223**, 415–420 (1982).
13. Bridges RS, Millard WJ. *Horm Behav* **22**, 194–206 (1988).
14. Bartke A. *J Reprod Fert* **11**, 203–206 (1966).
15. Bartke A, Lloyd CW. *J Endocr* **46**, 321–329 (1970).
16. Dixon WJ. *Statistical software.* Los Angeles: University of California Press, 1981: 112.
17. Holder AT, Wallis M, Biggs P *et al. J Endocrinol* **88**, 35–47 (1980).
18. Hughes JP, Elshotz HP, Friesen HG. *Endocrinology* **111**, 702–704 (1982).
19. Drago F, Van Ree JM, Bohus B *et al. Europ J Pharmacol* **72**, 249–253 (1981).
20. Esquifino AI, Villanua MA, Szary A *et al. Acta Endocrinol* **125**, 67–72 (1991).
21. Nicoll CS. *Handbook of Physiology*, Section 7: Endocrinology, vol. IV. American Physiological Society: Washington, 1974: 253.
22. Perkins NA, Westfall TC. *Neuroscience* **3**, 59–68 (1978).
23. Kempf E, Fuhrmann G, Thiriet G *et al. Neurochem Res* **10**, 969–981 (1985).

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