Mechanisms of precocious puberty induced in male rats by pituitary grafts*

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Summary. Male rats were grafted on Day 21 of age with ‘young’ (21 days old) or ‘adult’ (90 days old) pituitary glands and then treated daily with 4 mg bromocriptine/kg or vehicle. Plasma samples were obtained on Days 21, 25 and 35 and when balano-preputial separation occurred. Both types of grafts advanced the age at which balano-preputial separation occurred and increased prolactin concentrations. Bromocriptine treatment reduced the prolactin values in both grafted groups, but did not block the advancement of puberty in rats treated with ‘young’ pituitary grafts.

These results suggest the existence of two possible mechanisms in precocious puberty induced by pituitary grafts: one is prolactin-dependent (when ‘adult’ pituitary glands were used) and the other not directly related to prolactin (when ‘young’ pituitary glands were used).

Keywords: balano-preputial separation; pituitary graft; prolactin; bromocriptine; testosterone; rat

Introduction

It is well-known that hyperprolactinaemia induces precocious puberty in female (Advis & Ojdeda, 1978; Advis *et al.*, 1981) and male (de Jong & van der Schoot, 1979) rats. In female rats, puberty can be advanced by grafting a pituitary gland from an ‘adult’ donor (90 days old) and is associated with an increase in plasma prolactin concentrations and can be blocked by bromocriptine treatment. However, the advancement induced by grafting a pituitary gland from a ‘young’ donor (21 days old) was not associated with increased prolactin concentrations and cannot be blocked by bromocriptine. These results suggested the existence of two possible mechanisms in precocious puberty induced by pituitary gland grafts: one being prolactin-dependent and the other not directly related to prolactin (Gonzalez *et al.*, 1986).

In the present work we have tried to confirm whether these two possible mechanisms are present in male rats, using balano-preputial separation as the index of puberty (Korenbrodt *et al.*, 1977).

Materials and Methods

Animals. The animals used in this work were ‘true littermate’ male Wistar rats (Adler, 1986) born in our laboratory. They were kept under conditions of controlled light (12 h light:12 h darkness; lights on at 07:00 h) and temperature (20°C) and Sanders rat chow and tap water were available *ad libitum*. Litters born on the same day were marked and thoroughly mixed and returned to the mothers in groups of 8 per mother. Animals were weaned on Day 21 and housed in group cages (4–5 per cage).

Surgical procedures. Pituitary gland grafting was performed under light ether anaesthesia on Day 21. The pituitary gland of a littermate (‘young donor’) or an ‘adult’ donor (90-day-old brother) was transplanted under the right kidney capsule. Sham-operated animals grafted with a piece of adipose tissue instead of a pituitary gland served as controls. At autopsy the pituitary transplant was examined for size, colour and vascularity to confirm that it was functional.

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Bromocriptine treatment. Bromocriptine mesylate (Sandoz, Basle, Switzerland) was dissolved in saline (0.154 M-NaCl) with 10% ethanol. Beginning on Day 21, animals received bromocriptine at 4 mg/kg given as two injections at 10:00 and 22:00 h or vehicle daily. Injections were performed subcutaneously.

Experimental procedures. In Exp. 1, males (21 days of age) received a pituitary gland from a littermate or an 'adult' donor under the right kidney capsule. Animals were checked daily and the age and body weight of all the animals were recorded on the day of balano-preputial separation.

In Exp. 2, males (21 days of age) received a pituitary gland from a littermate or an 'adult' donor under the right kidney capsule. Immediately afterwards, bromocriptine (4 mg/kg) or vehicle treatment began. The age and the body weight on the day of balano-preputial separation were recorded in all animals. Blood samples were obtained before daily injections of bromocriptine or vehicle at 10:00 h on Days 21, 25, 35 and at decapitation performed on the day of balano-preputial separation. Samples were obtained by jugular venepuncture after light ether anaesthesia as described previously (Gonzalez *et al.*, 1986).

Hormonal assays. Plasma samples, obtained by centrifugation of blood for 20 min at 1650 g, were immediately frozen and stored at -20°C until assayed. Plasma prolactin concentrations were determined by a double radio-immunoassay method using a kit from NIAMDD (Bethesda, Maryland, U.S.A.). Rat-Prl-I-4 was labelled with ^{125}I by the chloramine T method (Greenwood *et al.*, 1963). The prolactin plasma values are expressed in ng/ml of the reference preparation Rat-Prl-RP-3. Testosterone was determined as previously described (Rodriguez-Padilla *et al.*, 1987). To avoid interassay variations, all samples were measured in duplicate, in the same assay. Intra-assay variabilities were respectively 9% and 5% for prolactin and testosterone, the limits of sensitivity being 0.1 ng and 5 pg/tube, respectively.

Statistics. Values are given as means $\pm$ s.e.m. Differences between groups were analysed using an ANOVA followed by a Duncan new multiple range test.

Results

Male rats grafted with 'young' pituitary glands showed precocious balano-preputial separation, occurring at a lower weight, than in sham-operated controls. Bromocriptine treatment blocked only the effect induced by 'adult' transplants (Table 1).

Table 1. Effects of bromocriptine (from the age of 21 days until the time of balano-preputial separation) on the precocious puberty induced by a 21-day-old (young) or a 90-day-old (adult) pituitary gland graft

Group	No. of rats	Age (days)	Body weight (g)
Experiment 1			
(1) Sham-operated	13	40.38 $\pm$ 0.34	142 $\pm$ 8
(2) 'Young' graft	15	36.46 $\pm$ 0.13*	116 $\pm$ 4*
(3) 'Adult' graft	15	36.80 $\pm$ 0.18*	120 $\pm$ 6*
Experiment 2			
(4) Sham-operated + vehicle	12	41.25 $\pm$ 0.26	148 $\pm$ 6†
(5) 'Young' graft + vehicle	11	39.00 $\pm$ 0.42*	133 $\pm$ 4
(6) 'Adult' graft + vehicle	8	38.37 $\pm$ 0.28*	125 $\pm$ 6
(7) Sham-operated + bromocriptine	10	40.90 $\pm$ 0.24	143 $\pm$ 4
(8) 'Young' graft + bromocriptine	12	37.91 $\pm$ 0.23*	112 $\pm$ 3
(9) 'Adult' graft + bromocriptine	9	40.66 $\pm$ 0.77**	133 $\pm$ 8

Values are given as means $\pm$ s.e.m.

* $P < 0.01$ vs Group 1 (Exp. 1) or Group 4 (Exp. 2); ** $P < 0.01$ vs Group 8; † $P < 0.01$ (grafted vs sham-operated animals) (ANOVA followed by Duncan new multiple range test).

Male rats grafted with an 'adult' or 'young' pituitary gland showed hyperprolactinaemia, the plasma prolactin concentrations being higher in animals grafted with 'adult' pituitary glands than in those grafted with 'young' pituitary glands. Bromocriptine treatment reduced the prolactin values in both types of animals (Fig. 1).

Fig. 1. Plasma prolactin concentrations in animals grafted on Day 21 of age with one 'adult' (■) or 'young' (●) pituitary or sham-operated (▲). Animals were treated until balano-preputial separation (BPS) with bromocriptine (4 mg/kg/day) (—) or vehicle (---). The numbers of animals were as indicated in Table 1 (Exp. 2). Values are expressed as means $\pm$ s.e.m. ^a $P < 0.01$ vs other groups; ^b $P < 0.01$ vs other groups (ANOVA followed by Duncan new multiple range test).

Plasma testosterone concentrations at balano-preputial separation were similar in rats sham-operated (1224 ± 319 pg/ml), or those grafted with 'young' (782 ± 150 pg/ml) or 'adult' (799 ± 149 pg/ml) pituitary glands. Bromocriptine treatment reduced plasma testosterone concentrations in all groups, although the effect was only significant ($P < 0.05$) in animals grafted with 'adult' pituitary glands (451 ± 55 vs 799 ± 149 pg/ml in similar animals treated with vehicle).

Discussion

The main objective of this work was to analyse the difference, if any, in the mechanism by which pituitary grafts of different donor age advanced puberty in male rats. Our results showed that prepubertal male rats grafted with one 'young' or 'adult' pituitary exhibited precocious balano-preputial separation. The degree of advancement was less than described by de Jong & van der Schoot (1979), perhaps due to the reduced number of pituitary glands grafted in our experiments.

The animals grafted with an 'adult' pituitary developed a greater increase in plasma prolactin concentrations than did those grafted with a 'young' pituitary gland. The low efficiency shown by 'young' pituitary glands in releasing prolactin when grafted has been previously described for females (Gonzalez *et al.*, 1986) and could be explained by their low prolactin content (Döhler *et al.*, 1977). Nevertheless, in spite of the different increases in prolactin concentrations, there was a similar advancement in puberty. At least two explanations are possible: (1) increases in prolactin concentrations above a 'threshold' induces the precocious puberty independent of the absolute plasma values and (2) other factors different from prolactin are involved in precocious puberty. The normal balano-preputial separation in sham-operated animals treated with bromocriptine

indicated that, even with the lowest concentrations of prolactin obtained by this treatment, puberty occurs at the physiological time.

In the animals grafted with 'adult' pituitary glands, the increase in plasma prolactin values and the blockade of precocious puberty after bromocriptine treatment suggested that prolactin release from the graft was responsible for the changes observed in balano-preputial separation. Assuming that the effect of bromocriptine is only on pituitary prolactin secretion (Richardson & Donatsch, 1985; Steger & Morgan, 1985), the inability of bromocriptine to block precocious puberty induced by 'young' grafts suggested that the effect is mediated by a factor other than prolactin.

Plasma testosterone concentrations at the time of balano-preputial separation were similar in rats in Groups 4, 5 and 6 (all vehicle treated), agreeing with data from de Jong & van der Schoot (1979) indicating that testosterone increased only in the early days after transplantation and was later normalized. Our results showing that bromocriptine treatment reduced plasma prolactin concentrations in animals grafted with 'adult' or 'young' pituitary glands below 5 ng/ml, but modified testosterone plasma levels only in animals grafted with 'adult' pituitary glands, suggested a different action of bromocriptine treatment on testicular function.

In conclusion, the present results suggest that pituitary grafts advanced puberty in males, as well as in females (Gonzalez *et al.*, 1986), by two possible mechanisms, one prolactin dependent and the other not directly related to prolactin, depending on the age of the pituitary graft. Our previous results (Aguilar *et al.*, 1987) suggested that, at least in females, precocious puberty induced by 'young' pituitary glands is mediated by an early secretion of gonadotrophins after grafting, since FSH and LH increased 12 h afterwards, and also that the effect on puberty can be blocked by the administration of specific LH antiserum or by grafting pituitary glands depleted of gonadotrophins.

Since an early secretion of gonadotrophins has also been described in prepubertal females grafted with 'adult' pituitary glands (Advis & Ojeda, 1979), a better interpretation of precocious puberty induced by pituitary grafts could be obtained by analysing the following: (a) the mechanism controlling gonadotrophin secretion from 'young' or 'adult' grafts; (b) the biological activity of gonadotrophins secreted, since different forms of LH with different biological and immunological activities have been reported for immature rats (ter Haar & Wilson, 1978; Buckingham & Wilson, 1985); and (c) the structure and biological actions of prolactin secreted by 'young' or 'adult' grafts.

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