Induction of superovulation in prepubertal female rats by anterior pituitary transplants

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Summary. Transplants of 26-day-old rats of an anterior pituitary gland from adult intact or castrated male, 20-day-old or adult ovariectomized female donors (all of which contained large amounts of FSH) resulted in superovulation in recipients on the morning of Day 29. Transplants of the gland from 20-day-old males and adult cyclic females could not advance the time of first ovulation or induce superovulation. In the rats in which superovulation could be induced, a marked increase in plasma FSH was noted in recipients shortly after transplantation and the high levels of plasma FSH were maintained until at least 12 h after grafting. These rats also showed preovulatory surges of LH and FSH 54 h after grafting. No obvious elevation of plasma FSH was noted over 72 h in recipients in which superovulation could not be induced.

These findings suggest that the final maturation of follicles for superovulation is induced by a transient release of a large amount of FSH from the grafted pituitary gland and that the sex of the pituitary donor has no bearing on this phenomenon.

Introduction

It is well documented that the timing of the first ovulation can be advanced in immature rats by the administration of pregnant mare serum gonadotrophin (PMSG) (Cole, 1936) or human chorionic gonadotrophin (hCG) (Selye & Collip, 1933). Wuttke & Gelato (1976) and Advis & Ojeda (1979) demonstrated that pituitary grafts under the kidney capsule were also capable of advancing the day of vaginal opening or the first ovulation in immature female rats. In our preliminary experiments, grafts of an anterior pituitary gland of adult male rats under the kidney capsule of immature female rats induced superovulation 3 days later, whereas the pituitary gland of adult female rats was not effective. The present study was undertaken to examine the mechanisms for the induction of superovulation by measuring plasma levels of FSH, LH and prolactin in immature rats with pituitaries transplanted under the kidney capsule.

Materials and Methods

Animals. Adult and immature rats of the Wistar strain raised in our laboratory were used. They were kept in conditions of controlled temperature and lighting (lights on 05:00–19:00 h).

Grafting of pituitary. Anterior pituitary glands were removed from healthy male or female rats at three different reproductive states, i.e. adult (90–120 days old), 3 weeks after gonadectomy (surgery at 70–90 days old) and immature (20 days old). One anterior pituitary gland was transplanted under the left kidney capsule of 26-day-old female rats between 09:00 and 11:00 h while the animals were anaesthetized with ether. Sham-operated controls underwent the same surgical procedure but a piece of adipose tissue was transplanted instead of a pituitary gland.

Effect of pituitary grafts on the first ovulation. The animals were then inspected twice each day for vaginal opening. Vaginal smears were taken daily after vaginal opening. All animals were killed by decapitation between 09:00 and 11:00 h on the first day of dioestrus (leucocyte type of vaginal smear) after vaginal oestrus (cornified cells in vaginal smears). The oviducts were examined for oocytes under a dissecting microscope.

Follicular development induced by pituitary grafts. To determine the ability of the antral follicles to ovulate in animals after grafting a pituitary gland, 10 i.u. hCG (2200 i.u./mg; Sankyo Zoki Co., Tokyo, Japan) dissolved in 0.2 ml saline (8.5 g NaCl/l), were injected into the tail vein of rats anaesthetized with ether 30 h after grafting. The animals were killed 20 h after hCG and the oviducts were examined for oocytes.

Pituitary contents of FSH, LH and prolactin in donor animals. Donor-type rats (5–13/group; adult, gonadectomized and immature) were killed by decapitation at 11:00 h. Anterior pituitary glands, removed immediately after death, were homogenized in 5 ml cold (4°C) saline. After centrifugation at 36 000 g for 30 min at 4°C, the supernatant fraction was obtained and stored at –20°C until assayed.

Effect of pituitary grafts on peripheral levels of LH, FSH and prolactin. Animals with pituitary gland grafts were decapitated at various times after the transplantation of a pituitary gland from intact adult male and female rats, ovariectomized females and immature male and female rats. Individual blood plasma samples were saved for the determination of FSH, LH and prolactin. At autopsy, uteri were weighed and oviducts were examined for oocytes under a dissecting microscope. The kidney capsules of rats with an ectopic pituitary were carefully examined for viable pituitary tissue with new vascularization. Data derived from animals with questionable, i.e. poorly or non-vascularized, transplants were not included.

Radioimmunoassay of LH, FSH and prolactin. Plasma and pituitary concentrations of FSH, LH and prolactin were measured using the NIAMDD-rat-FSH, LH and prolactin radioimmunoassay kits. The antisera used were anti-rat FSH serum 9, anti-rat LH serum 4 and anti-rat prolactin serum 4. Reference preparations used for standards were rat FSH-RP1, rat LH-RP1 and rat prolactin RP1. Preparations for iodination were rat FSH-I-5, LH-I-5 and prolactin I-3. In the FSH and LH assay 200 µl plasma were used whereas in the prolactin assay 25 and 50 µl were assayed. Duplicate plasma samples were assayed. The intra- and inter-assay coefficients of variation were, respectively, 2.8 and 4.1% for FSH, and 3.5 and 19.7% for LH, 2.5 and 20.4% for prolactin. The lower limits of assay sensitivity for FSH, LH and prolactin were 10 ng, 8 ng and 0.4 ng per tube, respectively.

Statistics. The significance of the difference between two means was tested by Student's *t* test, but when more than two means were compared, an analysis of variance was carried out and the significance of the difference between means was determined by Duncan's multiple range test (Steel & Torrie, 1960); a probability level of $P < 0.05$ was considered statistically significant.

Results

Effect of a pituitary graft on the first ovulation and initiation of follicular maturation

As shown in Table 1, the age at first ovulation was advanced and the number of oocytes ovulated markedly increased in all groups of rats except those with a pituitary gland from an adult cyclic female or an immature male.

Administration of hCG 30 h after grafting produced superovulation in all animals bearing a pituitary gland from 20-day-old females (25.8 ± 3.9 ova (s.e.m.), $N = 6$), ovariectomized females (33.2 ± 5.3 , $N = 5$), adult males (29.6 ± 7.1 , $N = 5$) or castrated males (65.8 ± 2.6 , $N = 5$). No ovulatory response to hCG was noted in sham-grafted animals ($N = 5$) and animals bearing a pituitary graft from adult cyclic females ($N = 5$) or 20-day-old male rats ($N = 5$).

Table 1. Age at the first ovulation and number of oocytes of immature rats receiving an anterior pituitary gland graft

Pituitary gland donor	No. of recipient animals	Age (days)	No. of oocytes
Control	5	36.8 ± 1.1	10.8 ± 1.0
Female			
20 days old	23	29.2 ± 0.1*	34.9 ± 4.4*
Adult, cyclic	5	37.8 ± 0.4	11.2 ± 0.7
Ovariectomized	9	29.0 ± 0.0*	42.1 ± 4.7*
Male			
20 days old	9	36.9 ± 0.6	9.1 ± 0.9
Adult	8	29.0 ± 0.0*	34.7 ± 3.5*
Castrated	7	29.1 ± 0.1*	66.4 ± 5.6*

Values are mean ± s.e.m.

* Significantly different from the value for sham-grafted controls ($P < 0.05$, Student's t test).

Pituitary contents of FSH, LH and prolactin in donor animals

FSH. The FSH content in the pituitary of castrated male rats was significantly higher than the values for all other groups. The lowest values were for the pituitaries of adult cyclic females and 20-day-old males (Table 2).

LH. Very high contents of pituitary LH were obtained in the gonadectomized animals (Table 2).

Table 2. Pituitary contents (µg/pituitary) of FSH, LH and prolactin in rats at various reproductive states

Pituitary gland donor	No. of animals	FSH	LH	Prolactin
Female				
20 days old	6	48.6 ± 3.3 ^b	128.1 ± 8.7 ^a	0.1 ± 0.04 ^a
Adult, cyclic	13	17.3 ± 1.4 ^a	143.1 ± 12.9 ^a	5.3 ± 0.6 ^c
Ovariectomized	8	129.9 ± 13.4 ^c	1769.0 ± 159.2 ^c	4.6 ± 0.8 ^c
Male				
20 days old	6	22.8 ± 1.8 ^a	45.7 ± 2.0 ^a	0.1 ± 0.04 ^a
Adult	5	130.6 ± 2.6 ^c	576.8 ± 21.5 ^b	2.1 ± 0.1 ^b
Castrated	5	201.5 ± 14.7 ^d	1495.7 ± 158.1 ^c	3.7 ± 0.6 ^{bc}

Values are mean ± s.e.m.

Within columns, values with different superscript letters are significantly different ($P < 0.05$, Duncan's multiple range test).

Prolactin. Values in 20-day-old male and female rats were significantly lower than those in all other groups (Table 2).

Changes in plasma concentrations of FSH, LH and prolactin after grafting a pituitary gland

In the sham-grafted rats, plasma FSH and prolactin did not change significantly throughout the period studied. All values of plasma LH in sham-grafted rats were below the sensitivity of the present assay (40 ng/ml).

In rats receiving a pituitary gland from an adult male, an ovariectomized female and a 20-day-old female, plasma FSH increased markedly within 3 h and high levels were maintained until 12 h (immature females) or 30 h (adult males or ovariectomized females) after grafting (Text-fig. 1a).

Values in these 3 groups rose significantly again at 54 h but were similar to control values by 60 h. A slight but significant increase in plasma FSH was observed by 6 h after grafting of a pituitary gland from an adult cyclic female whereas non-significant FSH elevation occurred in animals with a pituitary from a 20-day-old male rat (Text-fig. 1e).

Text-fig. 1. Plasma concentrations of FSH (a, e), LH (b, f), and prolactin (c, g) and uterine weight (d, h) in 26-day-old rats receiving pituitary gland grafts under the kidney capsule. The 3 groups represented in (a), (b), (c) and (d) are those in which superovulation can be induced. In (b) and (f) the dotted line shows the detection limit of the radioimmunoassay (40 ng/ml). Results are the means $\pm$ s.e.m. of 5 observations except where indicated.

Plasma levels of LH were greatly enhanced by 3 h after the transplantation of pituitary gland from adult male and ovariectomized female donors (Text-fig. 1b) but not by those from the other donors (Text-figs 1b & 1f). By 24 h plasma LH levels had returned to undetectable values in all

groups. In rats with a pituitary gland from an adult male or ovariectomized female, plasma LH increased markedly at 54 h but had fallen again by 60 h. In rats with a pituitary gland from a 20-day-old female rat, 4 out of 5 showed relatively low levels of plasma LH (80 ng/ml) at 54 h after transplantation, whereas 1 rat exhibited a very high value (889 ng/ml).

Plasma levels of prolactin were significantly increased by 3–6 h in all experimental groups (Text-figs 1c & 1g). In animals with a pituitary gland from an adult cyclic female, values were greatly enhanced 3 h after grafting. Although the levels declined by 12 h, the values were much higher than those in all the other groups and remained high throughout the rest of the period studied (Text-fig. 1g).

Changes in uterine weights after grafting of a pituitary gland

Uterine weights increased significantly up to 54 h in rats with a pituitary from an adult male or ovariectomized female (Text-fig. 1d). Although uterine weights also increased significantly in rats with a pituitary from a 20-day-old female, the values were much less than those of rats with pituitaries from adult male and ovariectomized female rats (Text-fig. 1d). Rats with a pituitary from an adult cyclic female or a 20-day-old male showed only slight increases of uterine weight (Text-fig. 1h).

Discussion

The present results demonstrate that the transplantation of a single pituitary gland with a high content of FSH under the kidney capsule could induce superovulation in immature female rats within 72 h. There was a positive relationship between the number of ovulations and the FSH content of the pituitary gland at the time of transplantation. Sex of the donor of the gland had no bearing on this response. Most of the follicles were already capable of ovulating at 30 h after grafting of the glands with a high content of FSH, suggesting that the magnitude and duration of the increase in plasma FSH within 24 h contributed greatly to the rapid maturation of follicles for superovulation.

After transplantation of a pituitary gland from 20-day-old females, plasma levels of LH in recipients did not increase within 30 h (Text-fig. 1b). However, superovulation could still be induced in these animals (Table 1), indicating that an increase in plasma LH concentrations after grafting a pituitary gland may not be involved in the final maturation of a large number of follicles. Nevertheless, the increases in uterine weight in females in this group were smaller than those of the other groups in which superovulation was induced (Text-fig. 1d). This may have been due to the lower levels of plasma LH and FSH after pituitary transplants causing reduced release of oestrogen, although the oestrogen secreted in all these groups may have been sufficient to trigger the surges of LH and FSH obtained 54 h after grafting a pituitary gland and leading to superovulation.

Superovulation can be induced in adult cyclic rats by the transplantation of a single pituitary gland from male and immature female rats under the kidney capsule or intramuscularly without any additional treatment with hCG and the failure of the adult female pituitary transplants to induce superovulation may be due to the insufficient content of FSH (Sameshima, Taya, Sasamoto & Etoh, 1982), as indicated by the similar results of the present experiments.

In the present study, in the rats in which superovulation occurred there was a small but significant increase in plasma prolactin concentrations 3 h after grafting the pituitary gland. It is unlikely that the occurrence of superovulation is induced by the hyperprolactinaemic conditions, because the pituitary grafts from adult cyclic female donors released significantly greater amounts of prolactin than did those from all other types of donors, but the time of vaginal opening and the first ovulation in these animals was not advanced. Induction of elevated serum levels of prolactin, by administration of exogenous prolactin (Clemens, Minaguchi, Storey, Voogt & Meites, 1969) or

by pharmacological induction of an increase in its endogenous release (Advis & Ojeda, 1978) leads to precocious puberty in the female rat. A direct stimulatory effect of prolactin on the hypothalamic levels to induce LH and FSH release has been suggested as one of the possible mechanisms by which the hormone advances the onset of puberty (Voogt, Clemens & Meites, 1969). However, Advis & Ojeda (1978) and Advis, Richards & Ojeda (1981) have indicated that the effect of prolactin on the onset of puberty is exerted by enhancing the responsiveness of the prepubertal ovary to the stimulatory effect of gonadotrophins, most probably by increasing the LH receptor content in the granulosa cells. A prolactin deficiency induced by the administration of bromocriptine before puberty in female rats resulted in decreased ovarian responsiveness to gonadotrophins and a marked delay in the onset of puberty (Advis, Smith White & Ojeda, 1981). The reason for the difference between previous reports and the present results on the effect of prolactin is not clear.

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