Pituitary and gonadal function in hypogonadotrophic hypogonadal (hpg) mice bearing hypothalamic implants*

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Summary. GnRH receptor values are 30–50% of normal in pituitaries of hpg male mice, and testicular LH receptors only 8% of normal (160.4 ± 17.6 and 2013 ± 208.1 fmol/testis respectively). In male hpg mice bearing fetal preoptic area (POA) hypothalamic implants for 10 days there was no change in pituitary GnRH receptors, pituitary gonadotrophin content, or seminal vesicle weight. However, testicular weights and LH receptors were doubled in 4/10 mice and 2 had increased serum FSH levels. Between 26 and 40 days after implantation pituitary GnRH receptors and pituitary LH increased to normal male levels, although at 40 days serum and pituitary FSH concentrations had reached only 50% of normal values. Testicular and seminal vesicle weights increased more than 10-fold by 40 days after implantation and LH receptors to 70% of normal.

In hpg female mice bearing hypothalamic implants for 30–256 days pituitary gonadotrophin concentrations were normal, even though GnRH receptors reached only 60% of normal values (6.1 ± 0.4 and 9.8 ± 0.4 fmol/pituitary respectively). Serum FSH was substantially increased from values of <30 ng/ml in hpg mice to within the normal female range in hypothalamic implant recipients. Ovarian and uterine weights increased after hypothalamic grafting from only 4–5% to over 74% of normal values. LH receptors increased from 6.5 ± 1.3 fmol/ovary for hpg mice to 566.9 ± 39.2 fmol/ovary for implant recipients. Vaginal opening occurred about 23 days after implantation and these animals displayed prolonged periods of oestrus.

Brain sections from animals with POA implants revealed that a prerequisite for restoration of near-normal pituitary and gonadal function was the anatomical connection of donor tissue to the median eminence of the recipient.

This study demonstrates that (1) implantation of fetal hypothalamic tissue largely reverses the GnRH deficiency of male and female hpg mice; (2) the pituitary–gonadal response to hypothalamic implantation follows a time-course similar to that observed during normal sexual maturation, but is more variable than the previously reported effects of multiple GnRH injections; and (3) although hypothalamic grafts in female hpg mice result in almost normal pituitary and ovarian endocrine function, cyclic ovarian activity does not occur, possibly reflecting the lack of appropriate neural control over the implanted tissue.

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Introduction

The failure of postnatal gonadal development in the hypogonadotrophic hypogonadal (hpg) mouse is the result of an inherited isolated deficiency in hypothalamic gonadotrophin-releasing hormone (GnRH) (Cattanach, Iddon, Charlton, Chiappa & Fink, 1977; Lyon, Cattanach & Charlton, 1981). The affected mice are characterized by undeveloped reproductive organs, low serum and pituitary gonadotrophin concentrations and a reduction in the concentration of pituitary GnRH receptors (Young, Speight, Charlton & Clayton, 1983). Nevertheless, differentiated but relatively inactive gonadotrophs have been identified in the pituitaries of hpg mice (McDowell, Morris & Charlton, 1982).

The pulsatile administration of low doses of GnRH to hpg male mice results in a rapid normalization of pituitary GnRH receptors, and serum and pituitary FSH content with associated elevations in pituitary LH content and increased testicular weight (Young et al., 1983; Charlton et al., 1983a). A similar regimen in hpg female mice resulted in pituitary and serum gonadotrophin concentrations typical of normal adult females after only 15 days of treatment (Charlton et al., 1983a). Similar results have been obtained by Belchetz, Plant, Nakai, Keogh & Knobil (1978) and Knobil (1980) with primates and by Marshall & Kelch (1979) who observed hormonal changes characteristic of puberty in humans with idiopathic hypogonadotrophic hypogonadism after pulsatile administration of GnRH.

Another way of providing chronic GnRH stimulation of the pituitary is the implantation of normal fetal mouse hypothalamus into the third ventricle of the adult mutant animal (Krieger et al., 1982). Grafts from appropriate areas of fetal brain have been shown to correct antidiuretic hormone deficiency in rats (Gash, Sladek & Sladek, 1980) and motor abnormalities of dopamine deficiency (Perlow et al., 1979; Bjorklund, Dunnett, Stenevi, Lewis & Iversen, 1980). In this study we have investigated the pituitary–gonadal endocrine changes that occur in female hpg mice bearing fetal hypothalamic implants, and have extended the initial study (Krieger et al., 1982) on hpg mice.

Materials and Methods

Animals. The hpg and normal male and female mice used in these studies resulted from crossing the F1 hybrid between two inbred strains, C3H/HeH and 101/H, and were aged 60–120 days. Animals were housed, in groups of 4–6, under lighting conditions of 14 h light (07:00–21:00 h)/24 h, with free access to food and water. At the end of the experiments the animals were bled by cardiac puncture. Pituitaries were rapidly removed, snap-frozen in liquid nitrogen, and stored at −70°C before GnRH receptor analysis, within 2 weeks. Serum was stored at −20°C until assayed. Testes and ovaries were trimmed of fat, weighed, snap frozen in liquid nitrogen, and stored at −70°C until measurement of LH receptors. Seminal vesicles and uteri were trimmed of fat and weighed.

Third ventricular implantation with preoptic area (POA) tissue. Donor tissue was taken from 16- to 18-day-old normal fetuses (both sexes) and the medial preoptic area was dissected out. The anterior border of the block of tissue removed was just caudal to the bifurcation of the cerebral artery, with the posterior border just anterior to the optic chiasma. The lateral cuts were made 0.5 mm from the midline and the depth of tissue was about 0.5 mm. POA tissue from 2 donors was introduced into the recipient's third ventricle, via a 22-gauge hypodermic needle with a stainless-steel plunger using the following stereotaxic co-ordinates with the incisor bar fixed 5 mm above the interaural line; 3.5 mm anterior to the interaural line, mid-line 5.5 mm down from the dura. For control implants the above procedure was repeated with fetal cortical tissue.

The brains were removed after decapitation and removal of the skull, fixed in Bouin's fluid, embedded in paraffin wax, and sectioned serially at 10 μm, stained with haematoxylin and eosin, and examined under the microscope to determine the nature and site of the graft.
**Pituitary GnRH receptor assay.** The GnRH agonist analogue (d-Ser (tBu)₆)des-Gly¹⁰-GnRH N-ethylamide (GnRH-A) used as the radioligand in the binding assay was kindly donated by Dr J Sandow (Hoechst AG, Frankfurt, West Germany). Iodination of GnRH-A, to specific activity of 1000–1500 µCi/µg, was performed as previously described (Young *et al.*, 1983). GnRH-A binding was assessed in an aliquant of an individual pituitary homogenate, under non-saturating conditions, by equilibration at 4°C with 50 000 c.p.m. ¹²⁵I-labelled GnRH-A (about 0·2 nm). Non-specific binding was measured in the presence of an excess of unlabelled GnRH-A (4 × 10⁻⁷ M) as previously described (Young *et al.*, 1983; Naik, Young, Charlton & Clayton, 1984). This method allows duplicate determinations of both total and non-specific binding on each pituitary. After correcting for protein content GnRH-A binding was expressed as fmol/pituitary (Young *et al.*, 1983).

**LH receptor assay.** HCG (CR121) was iodinated, to specific activity of 80 000 c.p.m./mg, using the lactoperoxidase method with subsequent purification on Sephadex G-100 and Sepharose-Concanavalin A as previously described (Catt, Ketelslegers & Dufau, 1976). HCG binding was measured in an aliquant of an individual mouse testis homogenate by equilibration at 22–24°C for 16 h with 50 000 c.p.m. ¹²⁵I-labelled hCG in the absence or presence of excess of unlabelled hCG (25 i.u.; Pregnyl: Organon) for assessment of non-specific binding. Incubations were terminated by dilution with 5 ml ice-cold isosmolar phosphate-buffered saline/0·1% bovine serum albumin (PBS/BSA), centrifugation, and aspiration of the supernatant. Radioactivity in the pellets was counted in a γ-spectrometer. Duplicate determinations of total and non-specific binding were obtained for each sample. HCG binding is expressed as fmol/gonad or fmol/mg gonadal tissue. Individual testes from normal mice were homogenized in 1 ml PBS/BSA whereas those from *hpg* mice (with and without implants) were homogenized in 0·5 ml. For HCG binding to ovaries from normal and hypothalamic-implanted animals, 1 ovary was homogenized in 0·45 ml PBS/BSA, whereas for *hpg* control animals 3 ovaries were pooled.

**Hormone assays.** Pituitary LH and FSH content and serum FSH concentrations were assayed by double-antibody RIA using rat hormone reagents supplied by the National Hormone and Pituitary Agency (Bethesda MD, USA) and results are expressed in terms of the respective RP-2 standards. Pituitary LH and FSH contents were measured in appropriate dilutions of individual pituitary homogenates and the results are expressed as µg hormone/gland. Dilutions of mouse pituitary homogenate gave parallel displacement curves to those obtained by using the rat LH and FSH standards. Inter- and intra-assay coefficients of variation for these assays were similar to those previously reported for measurement of small volumes of mouse serum (Young *et al.*, 1983) (8–10% and 3–5%, respectively). Lower limits (B/B₀ = 0·9) of detection for serum LH and FSH were 6 and 30 ng/ml, respectively and for pituitary LH and FSH were 0·32 and 1·3 µg/pituitary, respectively. When there was insufficient serum sample for measurement of both gonadotrophins we elected to measure FSH, rather than serum LH, in view of our previous findings (Young *et al.*, 1983) that multiple GnRH injections to *hpg* mice produced very rapid and dramatic rises in FSH while changes in serum LH could not be detected.

**Results**

**Effects of POA implants on the pituitary-testicular axis of *hpg* male mice**

In this experiment *hpg* male mice were killed 10, 26 or 40 days after implantation of fetal POA tissue. Control *hpg* animals bearing fetal cortical tissue were killed 25 days and 36 days after implantation.

Anatomical and histological assessment of grafts. Whenever pituitary and gonadal function had been stimulated donor tissue was present in the third ventricle closely apposed to the median eminence (Pl 1, Fig. 1). Three animals (2/10 in the 26-day and 1/10 in the 40-day POA-implanted
group) showed no change in any of the characteristics measured and data from these animals were excluded. Histological sections of the brains from these animals revealed no visible implant at the median eminence level of the third ventricle in 2, while in the other a small implant was visible at the top of the third ventricle, not in contact with the median eminence.

**Pituitary gonadotroph function.** Body weights of the hpg control, hpg fetal-cortex implanted, and hpg POA-transplant recipients did not differ significantly from those of age-matched normal males. Similarly, no differences in pituitary protein content (and by implication pituitary weight) was observed between POA transplant recipients and untreated controls (mean ± s.e.m. protein contents being 260-6 ± 15-8 µg/pituitary for normal male mice and 224-8 ± 20-7 and 274-7 ± 20-5 µg/pituitary for hpg males and hpg males + POA for 40 days, respectively, n = 9).

The GnRH-A binding to pituitaries of hpg mice was <50% of that of normal male littermates and transplantation of fetal cortex tissue did not affect this (Text-fig. 1a). No difference in any variable measured was found between untreated hpg mice and hpg mice bearing cortex implants for 25 or 36 days. The data for these two groups were therefore pooled. Hpg mice with POA implants for 10 days showed no change in pituitary GnRH-A binding (corrected for protein differences both between animals and groups), whereas between 10 and 40 days a gradual increase in GnRH receptors occurred until normal adult values were reached. Likewise, the pituitary content of LH and FSH 10 days after transplantation did not differ from control values but increased markedly at 26 and 40 days (Text-fig. 1c). At 40 days pituitary LH was within the range for normal adult male mice, although pituitary FSH reached only 44% of normal values. Serum FSH was unmeasurable (<30 ng/ml) in 8/10 POA-implanted animals at 10 days, being 109.5 and 169 ng/ml in the other two. Between 10 and 26 days serum FSH became detectable in all animals and reached almost 500 ng/ml by 40 days after implantation, although this value was only 50% of that found in normal adult male mice (Text-fig. 1b). The large standard errors for pituitary gonadotrophin and serum FSH measurements in POA-implanted animals (contrasted with the smaller errors for control and normal groups) are indicative of the variable effect of the implant in each individual. Serum LH was undetectable (<6 ng/ml) in all groups of hpg animals.

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**PLATE 1**

**Fig. 1.** Coronal section through the brain of a male hpg mouse 28 days after placement of a POA graft in the third ventricle. In this animal pituitary and gonadal function had been stimulated. The graft fills the ventricular lumen at this point and is in close contact with the median eminence (ME). × 75.

**Fig. 2.** Coronal section as above from a female hpg mouse 38 days after operation. In this animal pituitary, ovarian and uterine function had been stimulated. Again the graft fills the ventricle and is in contact with the median eminence. × 75.

**Fig. 3.** Coronal section at the level of the median eminence in a female hpg mouse 38 days after operation. The graft can be seen in the upper half of the third ventricle and made no contact with the median eminence. In this female, although pituitary function was stimulated, the ovaries and uterus were indistinguishable from the hypogonadal state. × 75.

**Fig. 4.** Coronal section at the level of the median eminence in a female hpg mouse 100 days after receiving a POA graft. The uterus of this female weighed 92.9 mg and the ovaries 9.3 mg. The animal was perfused with Zamboni's fixative (1-8% paraformaldehyde, 7.5% saturated picric acid in 0-1 m-phosphate buffer, pH 7-3) and the brain sectioned at 50 µm using a vibratome (Oxford Instruments). Sections were incubated for 48 h with an antibody to GnRH raised in rabbits (provided by Dr Robert Benoit of the Salk Institute) and the site of antibody binding was visualized using an anti-rabbit horseradish peroxidase conjugate (provided by Dr Neil Barclay, M.R.C. Cellular Immunology Research Unit, Oxford). Diamino benzidine (Aldrich Chemical Co. Ltd, Gillingham, Dorset, U.K.) was used as the chromogen. A rich plexus of GnRH-positive fibres can be seen at the base of the ventricle (arrow) with individual fibres crossing the median eminence. The control section in which synthetic GnRH was added to the first antibody incubation showed no staining of fibres. × 75.
(Facing p. 250)
Text-fig. 1. Effect of POA implants for 10–40 days on (a) pituitary GnRH-A binding, (b) serum FSH concentration, (c) pituitary FSH and LH content, (d) testicular hCG binding and (e) testicular weights in hpg male mice. Values are mean ± s.e.m. for 8–10 mice per group; individual values are also given in (d) and (e). n.d. = not detectable (<30 ng FSH/ml serum; <1·3 μg FSH-pituitary).

Testicular and accessory sex organ responses. In Text-fig. 1(d, e) testicular weights and hCG binding are shown for each animal to indicate the variable gonadal response to POA implantation. By 10 days after POA implantation testicular weight had doubled in 4/10 recipients, being the same as the control hpg mice in the other 6. By 26 and 40 days a much bigger increase in testicular weight occurred, with the lowest weight being 4 times that of hpg controls. The mean testicular weight after 40 days (32·6 ± 7·3 mg) was a third of that of normal adult mice and much greater than that of hpg mice (2·8 ± 0·1 mg). Seminal vesicle weights after cortex or 10-day POA grafts were no different from those of hpg controls (2·7 ± 0·4 mg). However, after POA implantation for 26 (26 ± 8·6 mg) and 40 days (45 ± 13 mg) seminal vesicle weights were increased, but only to a third that of normal adult values (142 ± 18 mg). Those animals with the lowest testicular weights 26 and 40 days after POA implantation also had the lowest seminal vesicle weights (not shown individually).
HCG binding in the testes of hpg mice was only 8% that in adult normal mice (160.5 ± 17.6 and 2013.1 ± 108.1 fmol/testis for hpg and adult testes, respectively). HCG binding in cortex-implanted hpg mice was similar to that in unimplanted controls (Text-fig. 1d). By 10 days after POA implantation HCG binding had increased to >5-fold that of hpg controls in the same 4 animals in which testicular weight was elevated (Text-fig. 1e) and by 26 and 40 days values were 55–70% of those of normal adults. When HCG binding was expressed per mg testis weight the data were less clear with mean values of 22.1 ± 1.3 and 57.2 ± 7.1 fmol/mg testis for normal and hpg mice respectively.

**Effect of POA implants on the pituitary–ovarian axis of hpg female mice**

Hpg female mice were killed 30–256 days after fetal POA implantation. Because of the different periods of transplantation, values for individual POA-implanted animals are presented, although when these were pooled as one group the variation (s.e.) was surprisingly small.

Anatomical and histological assessment of grafts according to vaginal status. Of the 13 POA-implanted female hpg mice 10 had a perforate vagina and an oestrous-type vaginal smear. Vaginal opening occurred between 13 and 41 days after POA implantation in these animals (average time to opening 23 days). Appropriate brain sections from all these 10 animals showed that the POA implant was in physical contact with the median eminence (Pl. 1, Fig. 2).

Immunoperoxidase staining for GnRH is shown in a representative brain section from an hpg female 100 days after POA transplantation (Pl. 1, Fig. 4). GnRH-immunoreactive nerve terminals are clearly visible in the zona externa of the median eminence adjacent to capillaries of the primary portal plexus. Although not examined at other times during the establishment of positive grafts of this study it is probable that GnRH-containing nerve fibres reached the median eminence much earlier than 100 days.

In the 3 mice, with implants for 38 days, in which vaginal opening did not occur (Mice 38B, 38D, 38E) there was no increase in uterine or ovarian weight (Table 1). At autopsy brain sections

**Table 1. Effect of implantation of the preoptic area (POA) in hpg mice**

<table>
<thead>
<tr>
<th>Time from implantation of POA (days)</th>
<th>GnRH-A bound (fmol/pituitary)</th>
<th>Pituitary LH (μg/pituitary)</th>
<th>Pituitary FSH (μg/pituitary)</th>
<th>Serum FSH (ng/ml)</th>
<th>Ovary wt (mg)</th>
<th>Uterine wt (mg)</th>
<th>hCG bound (fmol/ovary)</th>
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<td>30A</td>
<td>6.9</td>
<td>12.7</td>
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<td>131.0</td>
<td>1.20</td>
<td>54.1</td>
<td>467.9</td>
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<td>10.7</td>
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<td>204</td>
<td>1.87</td>
<td>25.0</td>
<td>351.2</td>
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<tr>
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Control values are mean ± s.e.m. for 10 mice/group except as indicated when 3 ovaries were pooled.

n.d., not detectable (<1.3 μg FSH/pituitary; <30 ng FSH/ml serum).

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revealed an implant of normal size at the top of the third ventricle but not in contact with the median eminence in one animal (Pl. 1, Fig. 3; Mouse 38D, Table 1), a small implant rostral to the median eminence in another animal (Mouse 38E), and a smaller than normal implant at the median eminence level in the third (Mouse 38B). Despite this there were minor endocrine changes in these 3 animals (Table 1).

**Pituitary gonadotroph function.** As previously reported (Young et al., 1983) GnRH-A binding in the pituitaries of female hpg mice was a third that of normal females at random stages of the cycle (2-9 ± 0-4 and 9-8 ± 0-4 fmol/pituitary, respectively). POA implants increased GnRH-A binding to 6-18 ± 0-4 fmol/pituitary, about 60% of normal values (Table 1: excluding Mice 38A, 38B, 38D, 38E). The reduced LH and FSH content of the pituitary of hpg females was normalized by effective POA grafts (Table 1). In hpg female mice all serum FSH values were <30 ng/ml, but POA implantation increased concentrations to the normal range for females in the majority of animals (Table 1) although in 3 animals it was <30 ng/ml.

As with the male animals there was insufficient serum for LH measurements and again FSH was selected as the serum hormone likely to provide the most sensitive index of pituitary gonadotroph stimulation. In Mice 38D and 38E (Table 1) which did not show vaginal perforation or change in uterine or ovarian weight, pituitary function was stimulated as indicated by increases to near-normal values of pituitary FSH and LH (3-3 and 2-8 μg/pituitary for FSH, and 14-2 and 9-2 μg/pituitary for LH), and serum FSH values of 234 and 62-8 ng/ml and ovarian hCG binding of 171-3 and 171 fmol/ovary. In the third animal of this type (Table 1, Mouse 38B) there was no change in pituitary and serum FSH, although pituitary LH content rose to 7-6 μg and ovarian hCG binding to 64-5 fmol/ovary.

**Ovarian and uterine responses.** The individual weights of both the ovary and the uterus were only 4-5% those of normal adults in hpg female mice (Table 1). The POA implant resulted in an average increase in ovarian and uterine weights of more than 15-fold to values 74% of that found in normal female mice. The hCG bound per hpg mouse ovary was <1% of that in normal adult ovaries and was markedly increased in ovaries from animals with effective POA implants, to 60% of normal values (Table 1).

**Discussion**

This study demonstrates that fetal hypothalamic tissue grafts partly reverse the GnRH deficiency of hpg mice of both sexes. However, the rate and magnitude of endocrine responses to the grafted tissue was variable and reflects variation in the rate at which the implanted tissue forms functional connections with the median eminence. A clear anatomical apposition or connection between grafts and the median eminence was evident at autopsy in all animals, male and female, in which activation of the pituitary–gonadal axis occurred. Close apposition was apparent 10 days after grafting in male hpg mice even though at this time endocrine responses were minimal, being limited to a small increase in testicular function in a few mice and occurring in the absence of any measurable pituitary response. Only if no graft was visible on brain sections, or if the graft was not in contact with the median eminence, was there failure of a pituitary–gonadal response. Indeed, even a few female animals with poorly-sited grafts showed some changes in ovarian function. This, in addition to activation of the pituitary before apparent neural outgrowth into the median eminence, suggests that the mechanism by which GnRH reaches the anterior pituitary in the first days or weeks after implantation may be by simple diffusion into the capillaries of the primary portal plexus, rather than by axonal transport of GnRH to this region, even though GnRH immunostaining may reveal fibre outgrowth as early as 10 days after implantation (A. J. Silverman, personal communication). By 60 days after transplantation both Thy 1 antigen (Charlton, Barclay & Williams, 1983b) and GnRH immunostaining (Krieger et al., 1982) clearly revealed fibre outgrowth from the grafts. GnRH-containing fibres were visible coursing through host tissue and
terminating in the proximity of capillaries of the primary portal plexus in the zona externa of the median eminence (Pl. 1. Fig. 4; Krieger et al., 1982). Although evidence of neuronal outgrowth from the grafted tissue was observed by 60 days in the female mice cyclic ovarian activity was not established in these animals, suggesting lack of appropriate neural control over GnRH secretion from the graft. Nevertheless, studies on the reproductive behaviour of POA-grafted females suggest that reflex ovulation may occur (Gibson et al., 1984; Charlton, Parry & Jones, 1985).

Although pituitary GnRH receptor concentrations and LH content in the pituitaries of hpg male mice with transplants were almost normal at 40 days after implantation, pituitary and serum FSH remained low. However, if comparison is made with normal 40-day-old male mice (Cattanach et al., 1977) rather than adults, a pattern of pituitary gonadotrophin levels similar to that in these 40-day grafted male hpg mice is found. Similarly, although seminal vesicle weights 40 days after POA transplantation were well below those found in normal adult animals of an age similar to that of the experimental hpg mice, they were almost identical to those found in 40-day-old normal male mice (see Cattanach et al., 1977). However, even at 40 days after grafting testicular weights were less than half those found in 40-day-old normal animals. The failure to stimulate testicular growth fully may partly be due to the lack of early FSH stimulation of the testes, because hpg mutants have never gone through normal puberty. This may explain why prolonged treatment with GnRH injections (Young et al., 1983; Charlton et al., 1983a), testosterone implants (Charlton et al., 1983a) or longer-term POA grafts (Krieger et al., 1982) have not so far produced a testis of normal adult size despite the stimulation of full spermatogenesis.

We have previously reported that low-dose pulsatile injections of GnRH in hpg males normalize pituitary GnRH receptors after only 3 days of treatment, with pituitary and serum FSH values reaching normal levels after 7 days, while pituitary LH content is only marginally elevated (Young et al., 1983; Charlton et al., 1983a). In these injection studies (Young et al., 1983) testicular weight increased to 4 times that of control hpg mice after only 7 days. The rapid rise in pituitary FSH content and testicular weight with a relative failure to stimulate an increased pituitary LH content contrasts with the slower time-course of pituitary and gonadal stimulation in the hypothalamus transplant recipients. Although no long-term study with pulsatile GnRH has yet been performed for comparative purposes, it appears that the POA implants may function in a more physiological manner. This is further indicated by the fact that the multiple GnRH injections caused gross changes in the morphology of the gonadotrophs of the anterior pituitary of hpg mice in that large numbers of lipid droplets accumulated in the cytoplasm (Megson, Lewis, Morris, Charlton & Fink, 1983; Morris & Charlton, 1983); this does not happen after POA grafting. Therefore, despite the fact that we cannot guarantee exactly how many GnRH neurones are transplanted or that they will be in the most propitious anatomical site to stimulate pituitary function, such grafts have produced the best overall physiological reversal of the hypogonadotrophic hypogonadism.

Although the female hpg mice were implanted with hypothalamic tissue for between 30 and 256 days, less variability in the overall physiological responses of these animals was observed. Even though pituitary GnRH receptors were not increased to normal adult levels, pituitary gonadotrophin content was normalized and serum FSH was stimulated to well above the normal range. Indeed, the POA grafts can promote a normal response of the entire pituitary–gonadal axis after only 30 days. As normal female mice have already reached adult status with regard to both pituitary LH and FSH content at 30 days of age the POA grafts appear to have rendered the hpg state normal over a similar time span as occurs during normal development. This normalization of hormonal measurements was also shown after multiple GnRH injections in hpg females (Charlton et al., 1983a), but again 40% of pituitary gonadotrophs contained lipid droplets after this treatment whereas grafts maintained normal gonadotroph ultrastructure (Morris & Charlton, 1983). In the 3 female hpg mice with wrongly positioned POA implants and/or reduced size of the POA implant, serum FSH values and ovarian hCG binding increased in the absence of a measurable effect on pituitary gonadotrophin content. Presumably, some GnRH from these implants stimulates minimal pituitary hormone secretion, but this is insufficient to elevate oestrogen levels as indicated.
by the lack of reproductile organ growth. Furthermore this 'reduced' GnRH amount does not stimulate pituitary GnRH receptors, suggesting that either greater exposure to GnRH is required or that oestrogen plays a role in receptor up-regulation.

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