Autonomous FSH synthesis in vitro in anterior pituitary glands and grafts from female rats

J. A. M. J. van Dieten, J. de Koning and G. P. van Rees

Division of Medical Pharmacology of the Center for Bio-Pharmaceutical Sciences, Sylvius Laboratory, P.O. Box 9503, University of Leiden, 2300 RA Leiden, The Netherlands

(Requests for offprints should be addressed to J. A. M. J. van Dieten)

REVISED MANUSCRIPT RECEIVED 12 NOVEMBER 1990

ABSTRACT

When pituitary glands from intact female, but not from ovariectomized rats, are incubated for 8 h in medium TC199 without further additives, FSH is synthesized. This LHRH-independent (or autonomous) FSH synthesis is prevented when bovine follicular fluid (bFF) is added to the incubation medium.

Results from preliminary experiments, however, indicate no clear autonomous FSH synthesis after long-term absence of LHRH. To investigate the regulatory mechanisms involved in autonomous FSH synthesis and release, pituitary glands (exposed to endogenous LHRH) and pituitary grafts (not exposed to endogenous LHRH) from intact and ovariectomized rats were incubated for 8 h in medium TC199. Total FSH content (FSH released plus FSH remaining in the tissue) was compared with that in non-incubated glands or grafts, giving an indication of FSH synthesis. In addition, some of the animals were given LHRH pulses for 40 h before incubation. When pituitary tissue was taken from intact female rats, FSH synthesis occurred in the animals' own glands and in grafts from LHRH-pretreated rats. No FSH synthesis was seen in ovariectomized rats with or without pretreatment with bFF and/or LHRH. However, when ovariectomized rats had been pretreated with oestrogen, FSH synthesis was measured in vitro after pulsatile LHRH treatment in vivo.

The results indicate that autonomous FSH synthesis in vitro is dependent upon previous (in vivo) exposure of the glands to both oestrogen and LHRH. Journal of Endocrinology (1991) 129, 27–33

INTRODUCTION

It has been shown that during incubation of pituitary glands from intact female rats in medium without further additives, synthesis and release of follicle-stimulating hormone (FSH) (but not of luteinizing hormone (LH)) occurs. This can be suppressed by addition of steroid-free bovine follicular fluid (bFF) to the medium, presumably thus exposing the glands to inhibin (Jenner, de Koning & van Rees, 1982). Synthesis of FSH and its secretion in vitro is therefore at least partly independent of the presence of LH-releasing hormone (LHRH). This is explained by assuming that FSH secretion consists of separate LHRH-dependent and LHRH-independent components, the latter being sensitive to the inhibitory action of inhibin (Jenner et al. 1982). This agrees with observations in vivo since administration of high doses of LHRH antagonists suppressed partly the FSH secretion in ovariectomized rats (Grady, Shin, Charlesworth et al. 1985), whereas the LHRH-independent part of the FSH secretion was suppressed by bFF (Charlesworth, Grady, Shin et al. 1984).

In contrast with this view, however, pituitary transplants under the kidney capsule (devoid of LHRH) showed no autonomous FSH secretion (van Rees & van Dieten, 1987). Moreover, in the oestrous cycle of the rat both inhibin (Vale, Rivier, Hsueh et al. 1988) and oestradiol (Nequin, Alvarez & Schwartz, 1979) levels first rise and then drop before the onset of the autonomous FSH surge. In the present study, a possible role for these hormones and LHRH in autonomous FSH synthesis and release was investigated.
MATERIALS AND METHODS

Animals
Female rats from the Wistar-derived colony held in this laboratory were kept in an animal room with lights on from 07.00 to 19.00 h and a temperature of 22 °C. They had free access to food and water. Two pituitary glands, removed immediately after death from 3-week-old female rats of the same strain, were transplanted under the kidney capsule of each rat. When ovariectomized rats were used as recipients, they were operated on 2 weeks before transplantation. Two weeks after transplantation, the glands were removed and incubated. The endocrine status of the grafted pituitaries in terms of responsiveness to LHRH reflects that of the host (van Dieteren, de Koning & van Rees, 1989).

Pretreatments

LHRH pulses
Animals treated with LHRH pulses received a cannula in the right external jugular vein under light ether anaesthesia. The cannula was connected to an infusion pump in such a way that the animals could move freely. The pump delivered 2-min pulses of 0.1 ml containing 100 ng LHRH/kg body weight every hour for 40 h before removal and incubation of the gland.

Oestradiol
Rats treated with oestradiol received on the day of transplantation a s.c. implantation with Silastic tubing (Dow Corning, Midland, MI, U.S.A.; 1-6 mm inner diameter × 3-2 mm outer diameter, effective length 2-5 mm) containing oestradiol (Organon International, Oss, The Netherlands; 100 µmol/l oil), which produced physiological oestradiol blood levels (Williams & Lipner, 1982). During LHRH-pulse treatment 50 µg oestradiol benzoate (in 0.2 ml oil) was injected s.c. twice (40 and 24 h before decapitation) to increase the responsiveness of the pituitary gland to LHRH (van Dieteren & van Rees, 1983).

Bovine follicular fluid (bFF)
Rats treated with bFF received i.p. injections of 1 ml steroid-free bFF 64, 48, 40, 24 and 16 h before incubation. This treatment maintains low FSH blood levels as described (Hermans, Debets, van Leeuwen & de Jong, 1981). The bFF was prepared from follicles between 1 and 2 cm in diameter, aspirated from ovaries collected from the local abattoir. The fluid was treated with charcoal as described by Welschen, Hermans, Dullaart & de Jong (1977).

Incubations
After decapitation, the animals' own glands and the transplants were removed, halved and placed separately in incubation flasks containing 1 ml ice-cold medium TC199 (Boehringer, Mannheim, F.R.G.). Pituitary glands in situ were excluded from the experiment when no pituitary tissue was found under the kidney capsule at autopsy. This was the case in about 25% or 50% of the ovariectomized or the intact rats respectively. After a preincubation period of 30 min the medium was exchanged for an equal volume of fresh medium (without or, in some cases, with 10 µl bFF). In each experimental series a group of pituitary glands in situ and grafted glands were not further incubated but used for extraction of FSH in saline (non-incubated groups) to estimate the initial pituitary FSH content. After an incubation period of 8 h at 37 °C under 95% O2:5% CO2 with continuous shaking, the media were collected. The pituitary tissues were weighed and homogenized and FSH was extracted in saline. The media and the extracts were frozen until assay of FSH.

Synthesis of FSH was established by comparing total FSH after incubation (i.e. the amount released plus that remaining in the tissue) with the FSH content of non-incubated tissue.

Experimental design
To investigate the in-vivo conditions underlying the subsequent, autonomous, FSH release in vitro, pituitary glands in situ exposed to endogenous LHRH and grafted pituitary tissue (devoid of LHRH) were incubated after different treatments in vivo. The involvement of LHRH was established by pulsatile treatment of the rats with LHRH and that of the ovaries by ovariectomy whether or not followed by substitution with oestradiol or bFF. Note that the in-situ pituitary gland and the grafted tissue of the same rat were both incubated under similar conditions or were not incubated. The following experimental series was carried out.

Experiment 1 consisted of 38 intact rats (glands in situ exposed to relatively low endogenous LHRH). They were treated with saline (control, n = 19) or with LHRH pulses (n = 19). At autopsy, pituitary tissues from each treatment were divided into three groups. Group 1 was not incubated (n = 6 and 7, from control and LHRH-treated groups respectively), whereas groups 2 and 3 were incubated in medium alone (both n = 7) or in medium with bFF (n = 6 and 5 respectively).

Experiment 2 consisted of 31 ovariectomized rats (glands in situ exposed to relatively high endogenous LHRH). They were treated with saline (control, n = 22) or with LHRH pulses (n = 9). At autopsy,
pituitary tissues from each treatment were divided into two groups. Group 1 was not incubated \((n=13\) and 4, from control and LHRH-treated groups respectively), whereas group 2 was incubated in medium alone \((n=9\) and 5 respectively).

Experiment 3 consisted of 24 ovariectomized rats. They were all pretreated with bFF and in addition with saline (control, \(n=12\)) or with LHRH pulses \((n=12)\). At autopsy, pituitary tissues from each treatment were divided into two groups. Group 1 was not incubated \((n=7\) and 6, from control and LHRH-treated groups respectively), whereas group 2 was incubated in medium alone \((n=5\) and 6 respectively).

Experiment 4 consisted of 41 ovariectomized rats. They were all pretreated with oestrogen and in addition with saline (control, \(n=23\)) or with LHRH pulses \((n=18)\). At autopsy, pituitary tissues from each treatment were divided into three groups. Group 1 was not incubated \((n=9\) and 6, from control and LHRH-treated groups respectively), whereas groups 2 and 3 were incubated in medium alone \((n=9\) and 6 respectively) or in medium with bFF \((n=5\) and 6 respectively).

Radioimmunoassay of FSH

The assay was essentially the same as described by Welschen, Osman, Dullaart et al. (1975), except that the antibody-antigen complex was separated from the free antigen by adsorption to Saccel (donkey anti-rabbit antibody-coated cellulose suspension; Innogenetics Ltd, Antwerp, Belgium). Specific antiovine FSH was a generous gift from Drs J. Dullaart and J. Th. J. Uilenbroek (Erasmus University, Rotterdam, The Netherlands). Rat-FSH-I-6 and FSHRP-1, kindly provided by the NIADDK (Bethesda, MD, U.S.A.), were used for iodination and as standard respectively. The sensitivity of the assay, defined as the amount of standard required to suppress binding of iodinated FSH to 85% of the amount occurring in the absence of unlabelled hormone, was estimated as 5-8 ng/tube. The intra- and interassay coefficients of variation were 5-5% and 16% respectively.

Statistical analysis

Statistical comparisons were made by analysis of variance followed by Duncan’s multiple comparison test (Steel & Torrie, 1960). A difference was considered to be significant when analysis of variance showed significant heterogeneity for the whole group and the multiple comparison test gave a value of \(P<0.05\) for the two groups concerned.

RESULTS

Experiment 1: Intact rats (Fig. 1)

The pituitary glands in situ showed, whether or not pretreated with LHRH, a significant synthesis of FSH during incubation in medium only (group 1A versus 2A and 1B versus 2B). This synthesis was prevented by addition of bFF to the medium (2A versus 3A and 2B versus 3B).

The grafted pituitary glands showed a non-significant increase in total FSH after incubation (1A versus 2A). However, pretreatment with LHRH in vivo induced significant FSH synthesis (group 1B versus 2B) which was suppressed by adding bFF to the medium (2B versus 3B).

Hence, the presence of endogenous or exogenous LHRH seems to be a prerequisite for new, autonomous, synthesis of FSH in vitro that can be blocked by factors present in bFF (presumably inhibin).

Experiment 2: Ovariectomized rats (Fig. 2)

In contrast to intact rats, pituitary glands in situ (group 1A versus 2A and 1B versus 2B) and grafted glands (group 1A versus 2A and 1B versus 2B) from ovariectomized rats showed no FSH synthesis in vitro, regardless of in-vivo treatment with pulsatile LHRH.

Thus, besides LHRH, presumably ovarian factors such as inhibin and/or oestradiol are necessary for autonomous FSH synthesis in vitro. In the following experiments, their effectiveness in vivo on subsequent autonomous FSH synthesis and release in vitro was studied.

Experiment 3: bFF-treated ovariectomized rats (Fig. 3)

Pituitary glands in situ (group 1A versus 2A and 1B versus 2B) or grafts (group 1A versus 2A and 1B versus 2B) from ovariectomized rats which had been injected with bFF, and whether or not pretreated with pulsatile LHRH, showed no significant FSH synthesis during subsequent incubation.

Hence, inhibin in vivo is not directly involved in the process leading to autonomous FSH synthesis in vitro.

Experiment 4: Oestradiol-treated ovariectomized rats (Fig. 4)

Pituitary glands in situ and grafts from ovariectomized rats (groups 1A versus 2A) pretreated with oestrogen showed no FSH synthesis in vitro, whereas pretreatment with pulsatile LHRH (groups 1B versus 2B) did. In the in-situ pituitary gland (group 2B versus 3B) this synthesis was suppressed by addition of bFF to the media. The results indicate that, in addition to LHRH, oestrogen in vivo is necessary to
cause autonomous FSH synthesis during subsequent incubation.

**Summary of plasma FSH levels**
The oestradiol and bFF pretreatments resulted in significantly ($P<0.05$) decreased serum FSH levels compared with control (ovariectomized) rats (respectively $1615 \pm 93$ ($n=11$), $1230 \pm 127$ ($n=8$) and $2258 \pm 109$ ($n=17$) µg FSH-RP-1/l). After additional LHRH treatment these levels were $2391 \pm 170$ ($n=9$), $1091 \pm 102$ ($n=8$) and $2896 \pm 173$ ($n=15$) respectively. These results indicate that LHRH significantly

*Journal of Endocrinology (1991) 129, 27–33*
FIGURE 3. Total FSH (amount of FSH released in vitro plus that remaining in the pituitary tissue) of pituitary glands in situ and grafts from bFF-pretreated ovariectomized rats. After pulsatile treatment in vivo with (A) saline or (B) LHRH, the pituitary tissues were divided into two groups: group 1 was not incubated (open bars) whereas group 2 was incubated in medium alone (hatched bars). Values are means ± S.E.M. (n = 5-7).

FIGURE 4. Total FSH (amount of FSH released in vitro plus that remaining in the pituitary tissue) of pituitary glands in situ and grafts from oestradiol-pretreated ovariectomized rats. After pulsatile treatment in vivo with (A) saline or (B) LHRH, the pituitary tissues were divided into three groups: group 1 was not incubated (open bars) whereas group 2 was incubated in medium alone (hatched bars), and group 3 in medium with bFF (cross-hatched bars). Values are means ± S.E.M. (n = 5-9). *P<0.05 compared with non-incubated glands in the same pretreatment group; **P<0.05 compared with non-incubated glands and with glands incubated with bFF in the same pretreatment group (Duncan’s multiple comparison test).
increased plasma FSH levels in control ovariectomized rats and oestradiol-treated rats. In intact rats, LHRH treatment did not significantly increase plasma FSH levels: 323 ± 60 (n = 18) and 218 ± 49 (n = 16) (µg FSH-RP-1/l) respectively. The data presented here were analysed by Duncan's multiple comparison test.

### DISCUSSION

In agreement with the results of a previous study (Jenner et al. 1982), incubation of an intact rat's own pituitary gland resulted in a significant synthesis of FSH which could be suppressed by bFF, which presumably contains inhibin. Incubation of pituitary tissue transplanted 2 weeks earlier under the kidney capsule, however, revealed no significant synthesis of FSH. Only the pituitary in situ would have been exposed to endogenous LHRH. Therefore stimulation of the FSH synthesis/release mechanism by endogenous LHRH in vivo might underlie the subsequent, autonomous, FSH synthesis and release during incubation of the pituitary gland. To test this hypothesis, pituitary graft-bearing intact female rats were given pulses of LHRH during the last 40 h before incubation. The validity of this approach has been shown before, since in hypophysectomized rats bearing autotransplants this treatment induced plasma FSH levels which were elevated five-fold over controls (van Rees & van Dieten, 1987). Incubation of the in-situ glands gave the same results as those of rats which had not been given LHRH. However, the grafts now showed significant FSH synthesis in vitro which could be suppressed by bFF. The results, therefore, indicate that previous in-vivo exposure of the pituitary glands to LHRH is
necessary for subsequent autonomous FSH synthesis and release. However, this role for LHRH could not be demonstrated when ovariectomized pituitary graft-bearing rats were used. Therefore it was concluded that, in addition to LHRH, ovarian secretory products might also be involved in the processes underlying autonomous FSH synthesis and release in vitro.

Two major ovarian factors involved might be oestradiol and inhibitin, since their blood levels rise and fall before the onset of the LHRH-independent FSH surge on the night of pro-oestrous. Ovariectomized rats were therefore treated with oestrogen or bFF and with or without pulsatile LHRH to investigate to what extent these (interrupted at autopsy) treatments affected autonomous FSH synthesis and release in vitro. The results show that treatment with bFF did not result in in-vitro synthesis of FSH. Only the combined treatment with oestrogen and pulsatile LHRH showed a significant FSH synthesis which could be suppressed by bFF in vitro. Previous in-vivo exposure to oestrogen and LHRH might therefore be necessary for in-vitro synthesis of FSH.

Pulsatile administration of LHRH increased the initial FSH contents of both the in-situ glands and the grafts only from otherwise untreated ovariectomized rats. Therefore ovarian secretory products may have an inhibitory effect on the in-vivo reaction to LHRH pulses. The inhibitory effects of inhibitin and oestradiol are also demonstrated by the low initial FSH contents of the in-situ glands of intact female rats and of bFF- or oestradiol-treated ovariectomized rats compared with ovariectomized rats. In the transplants, oestrogen had no clear inhibitory effect on initial FSH contents. This agrees with the hypothesis that the inhibitory effect of oestrogen on pituitary FSH contents is through suppression of endogenous LHRH secretion.

In conclusion, in-vitro FSH synthesis, which could be suppressed by bFF, was found to be dependent upon previous in-vivo exposure to both LHRH and oestrogen. The precise mechanism of action underlying autonomous FSH synthesis and release is still not known, but an absolute distinction between autonomous and LHRH-dependent FSH synthesis and release as proposed previously (Jenner et al. 1982) might not be valid anymore.

REFERENCES