Regulation of gonadotrophin secretion by inhibin, testosterone and gonadotrophin-releasing hormone in pituitary cell cultures of male monkeys

U Fingscheidt, G F Weinbauer[1], H L Fehm and **E Nieschlag[1]**

Medical Clinic I, Medical University of Lübeck, D-23538 Lübeck, Germany and [1]Institute of Reproductive Medicine of the University, D-48129 Münster, Germany

(Requests for offprints should be addressed to U Fingscheidt, Medical Clinic I, Medical University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany)

Abstract

The effects of bovine inhibin, testosterone and GnRH on gonadotrophin secretion by primate pituitary cells were characterized *in vitro* using pituitaries from six male rhesus monkeys and one male cynomolgus monkey. The effect of inhibin on basal secretion of FSH and LH was investigated. Dose–response curves in monkeys and rats were compared. GnRH dose–response curves in the presence and absence of testosterone were also examined in monkeys.

In monkey pituitary cells, testosterone at a concentration of 10^{-7} M had no effect on LH or FSH secretion. Inhibin suppressed FSH secretion to 50·8% of that of controls with no effect on LH. In rats, FSH secretion was suppressed to 45·0% of that of controls with a median effective dose (ED_{50}, 95% range) of 1·298 (1·064–1·584) U/ml, compared with 1·024 (0·7204–1·455) U/ml in monkeys. In monkey pituitary cells, LH release was stimulated 9·9-fold and FSH 3·3-fold by GnRH. Testosterone had no effect on basal or GnRH-stimulated gonadotrophin release. These results support the view that the pituitary is not the target organ for the negative feedback action of testosterone in the male. *In vitro*, inhibin is the major regulator of FSH secretion at the pituitary level.

Journal of Endocrinology (1998) **159,** 103–110

Introduction

According to the classical inhibin hypothesis, secretion of follicle-stimulating hormone (FSH) is regulated by a dual feedback system of two testicular hormones: inhibin and testosterone. The main biological action of inhibin is the suppression of gonadotrophins, predominantly FSH, at the pituitary level. Since inhibin has become available in purified or recombinant preparations (Mason *et al.* 1985*a,b*, Forage *et al.* 1986, Mayo *et al.* 1986), this effect on FSH has been proved in different *in vitro* systems of cultured pituitary cells of rats (Scott *et al.* 1980, Farnworth *et al.* 1988) and sheep (Tsonis *et al.* 1986). Later, inhibin was administered *in vivo* to rats (Rivier *et al.* 1991), sheep (Mercer *et al.* 1987) and monkeys (Stouffer *et al.* 1994) and the suppressive action on FSH confirmed. A recent study based on the experimental model of the hypophysiotropic clamp, which eliminates feedback effects other than those at the pituitary site, suggested that suppression of FSH is mediated by inhibin in monkeys and that testosterone has no effect on the pituitary gland (Majumdar *et al.* 1995). Although there is little doubt about the effect of inhibin on FSH, direct proof that it acts at the pituitary cell in primates is lacking. We therefore established a cell culture system for monkey cells to characterize the effect of inhibin on pituitary cells *in vitro*. Furthermore, we repeated the experiment with rat pituitary cells to define possible interspecies differences between rats and monkeys with regard to the action of inhibin.

The control of FSH by inhibin is supplemented by the negative feedback action of testosterone on FSH. The degree to which either the hypothalamus or pituitary contribute to the effect of testosterone has not been defined thoroughly. Studies in different experimental models and different species have led to conflicting results. Dubey *et al.* (1987) showed that neither testosterone nor oestradiol had any negative feedback action at the pituitary in monkeys *in vivo*. *In vitro*, on the other hand, a suppressive effect of testosterone on luteinizing hormone (LH) secretion of pituitary cells could be demonstrated in rats (Kamel *et al.* 1987). Studies in men with hypothalamic hypogonadism treated with pulsatile gonadotrophin-releasing hormone (GnRH) confirmed this suppressive effect of testosterone on LH and FSH at the pituitary site (Bagatell *et al.* 1994). In another model of hypothalamus–pituitary-disconnected rams, both testosterone and inhibin decreased FSH in the serum under stimulation with GnRH (Tilbrook *et al.* 1993).

Our cell culture system was also used to characterize the effects of testosterone on basal and GnRH-stimulated

Journal of Endocrinology (1998) **159,** 103–110 *Printed in Great Britain*
0022–0795/98/0159–0103 $08.00/0

gonadotrophin secretion of primate pituitary cells *in vitro*.

Materials and Methods

Animals

Six intact adult male rhesus monkeys (*Macaca mulatta*) and one adult male cynomolgus monkey (*Macaca fascicularis*) were used for this study. The mean ± S.D. body weight of the rhesus monkeys was 12·6 ± 1·6 kg and the weight of the cynomolgus monkey was 4·7 kg. The animals were maintained in a controlled environment, with a 12 h light/12 h darkness photoperiod as described previously (Weinbauer *et al.* 1984). Pelleted monkey diet supplemented with fresh fruit was provided twice daily, and tap water was available *ad libitum*. Testosterone and bioactive LH as well as testicular volume, body weight and general health of the animals were normal.

A total of 60 adult male Wistar rats weighing 180–220 g were housed in cages (five animals/cage) under controlled temperature (23 °C) and lighting conditions (12 h light/12 h darkness) with free access to pelleted food and tap water.

Maintenance and handling of monkeys and rats complied with the German Federal Law for Care and Use of Laboratory Animals.

Preparation of pituitaries

In monkeys, five independent preparations of pituitaries were carried out. The first three were from individual animals, one cynomolgus and two rhesus monkeys. In each of the other two experiments, pituitary cells from two rhesus monkeys were pooled to increase the number of wells for different doses of substances tested. In addition, three independent experiments in rats with 20 rats per preparation of pituitary cells were carried out. The experiments described required two rats per experiment. To minimize the interexperimental variation, 20 rats were used in every preparation. Remaining cells were used for calibration of inhibin standards and for a study on the effect of basal GnRH on gonadotrophins (results not shown). Monkeys were anaesthetized with ketamine hydrochloride (20 mg/kg; Ketavet; Parke-Davis, München, Germany) and killed with CO_2. Rats were killed with CO_2 and decapitated. Pituitaries were removed immediately after death and transported at 37 °C in sterile PBS containing 100×10^3 U/l penicillin (Serva, Heidelberg, Germany), 100 mg/l streptomycin (Serva), 2·7 g/l glucose and 0·3% BSA (Behring, Marburg, Germany). A period of 15 min elapsed between the death of the animals and the beginning of cell preparation under a laminar flow hood.

Cell culture

Cells were prepared under sterile conditions as described previously (Hyde *et al.* 1982), with modifications. Briefly, pituitaries were rinsed in PBS and cut into approximately 1 mm^3 pieces using a scalpel. Incubation with trypsin (Type III; 1·5 g/l; Sigma, München, Germany) and DNase (Type I; 100 mg/l; Sigma) in PBS was carried out for 30 min at 37 °C, followed by a further incubation with DNase (200 mg/ml) in PBS for 4 min and EDTA (2×10^{-3} M) in PBS without Ca^{2+} and Mg^{2+} and for 5 min. Pituitary cells were then dispersed mechanically by repeated aspiration with a Pasteur pipette in DNase (100 mg/l) in PBS without Ca^{2+} and Mg^{2+}. This process was repeated 7 times for rat cells and 20 times for monkey cells. The dispersion was followed by centrifugation at 200 ***g*** for 10 min and resuspension in Dulbecco's modified Eagle's medium (DMEM) containing 100×10^3 U/l penicillin, 100 mg/l streptomycin, 1·2 g/l $NaHCO_3$, 10 ml non-essential amino acid solution (Serva), 1×10^{-3} M L-glutamine (Serva) and 10% fetal calf serum (Sigma) which had been pretreated with charcoal and dextran to remove steroids. Cells were counted, and viability was determined by measuring trypan blue exclusion. The cell suspension was diluted to 100×10^6 cells/l and distributed on 24-well culture plates (Costar, Cambridge, MA, USA) with 50×10^3 cells/well. The culture medium volume was 500 µl/well. Hormone levels were multiplied by a factor of 20 to convert units from ng/50×10^3 cells to ng/10^6 cells. Cell preparation was followed by a preincubation for 48 h at 37 °C, 98% humidity, 5% CO_2 in air. After 48 h the medium was changed, and, in six wells, the cells were detached by incubation with EDTA in PBS without Ca^{2+} and Mg^{2+}, and stained with trypan blue to determine viability. The same procedure was carried out after collection of medium at the end of the experiments. In addition, media collected from six dishes were centrifuged at 200 ***g*** for 10 min and resuspended in PBS without Ca^{2+} and Mg^{2+}, stained with trypan blue and examined microscopically. Media were collected and stored at − 20 °C until determination of FSH and LH by RIA.

Experimental protocol

Effect of inhibin on FSH and LH secretion in monkeys and rats The effect of incubation with inhibin in increasing doses on basal FSH and LH release was studied during an incubation period of 48 h. Highly purified bovine inhibin with a molecular mass of 31 kDa (D M de Kretser, Clayton, Vic, Australia) was dissolved after methanol precipitation in DMEM, and added to cell cultures in triplicate in doses from 0 to 20 U/ml after the preincubation for 48 h. Inhibin had a specific activity of 750 U/µg, and the concentration can thus be converted from U/l to nmol/l using the approximation: 1 U/l ≈43 nmol/l (Robertson *et al.* 1986). After 48 h, medium was removed and frozen for assay of hormones. This experiment was carried out once in a cynomolgus monkey cell culture, three times in rhesus monkey cell culture, and three times in rat pituitary cell cultures.

Effects of testosterone on basal and GnRH-stimulated secretion of FSH and LH in monkeys

The aim of this experiment was to determine the effects of testosterone on GnRH-stimulated secretion of gonadotrophins. After the preincubation of 48 h, culture medium was removed and replaced with warm fresh medium containing either 0·2% ethanol or testosterone (10^{-7} M; Sigma) in 0·2% ethanol. In three aliquots taken at the end of the experiment, testosterone was measured by luminescence assay and found to be $0{\cdot}98 \times 10^{-7}$ M. After a further 44 h, medium was replaced with one of the same composition (testosterone in ethanol or ethanol alone), and graded doses of GnRH (0, 10^{-11}, 10^{-9}, 10^{-7} and 10^{-6} M; Relefact LHRH; Hoechst, Frankfurt, Germany) were added in 10 µl PBS. After 4 h incubation with GnRH, medium was collected and stored for assay of hormones. This experiment was performed in triplicate and repeated twice.

Hormone analysis

Inhibin in the serum was measured in a double-antibody RIA validated for cynomolgus monkey, rat, human and bovine serum and follicular fluid (Fingscheidt *et al.* 1989). This RIA detected the 59 and 31 kDa forms of dimeric inhibin and also reacted with the α-subunit precursor protein pro-α C. Serial dilutions of rhesus monkey serum with a maximum volume of 100 µl serum/tube were parallel to those of cynomolgus monkey serum. Samples were assayed in duplicate using 50 µl serum/tube. The minimum detectable dose was 0·2 U/ml; the intra-assay coefficient of variation was 5·5% at 0·35 U/ml.

Monkey FSH in the medium was measured by a human FSH (NIAMDD hFSH-2):anti-ovine-FSH (H 31, NICHD) RIA system that employs a purified cynomolgus FSH preparation (WP-XV-104 C) as standard as described previously (Khan & Diczfalusy 1983). The detection limit was 0·2 ng/ml, the interassay coefficient of variation (n=6, at 1·2 ng/ml) was 8·2% and the intra-assay coefficient of variation was 6·5% at 1·2 ng/ml. Serial dilutions of the medium paralleled those of the standard preparation.

Monkey LH in the medium was measured by a heterologous RIA using a cynomolgus LH preparation (WP-XV-63–2429) as tracer, anti-human chorionic gonadotrophin as antibody and LER 1909–2 as standard, as described previously (Kurshid *et al.* 1991). The detection limit of this assay was 0·3 ng/ml, the interassay coefficient of variation (n=6, at 2·2 ng/ml) was 6·9% and the intra-assay coefficient of variation was 5·8% at 2·2 ng/ml. Serial dilutions of the medium paralleled those of the standard preparation.

Rat FSH (rFSH) and rat LH (rLH) in the medium were measured using double-antibody RIAs based on reagents supplied by NIDDK rFSH:rFSH-S-9 and rLH:rLH-S-9, standards: RP-2, anti-rabbit globulin (donkey) second antibody RD-17 (Wellcome, Dartford, Kent, UK), by the method of Solano *et al.* (1979). The minimum detectable doses were 3 ng/ml (FSH) and 1 ng/ml (LH), interassay coefficients of variation were 7·2% (rFSH) and 6·1% (rLH), and intra-assay coefficients of variation were 4·3% (rFSH) and 5·1% (rLH).

Testosterone was measured by a luminescence immunoassay developed in our institute (Kreysing & Nieschlag 1987). The detection limit of the assay was 0·27 mM, and intra-assay variation was 5·3%.

Statistical analysis

Each dose in the cell culture experiments was tested in triplicate, and medians of triplicate doses were calculated. For graphic representation of the data, means and standard deviation were computed from the three medians obtained from independent experiments.

Dose–response curves were fitted for the calculated medians of each of three identical experiments using the GraphPad Prism program to obtain values for median effective dose (ED_{50}), maximal suppression or stimulation (bottom or top) and 95% ranges. Curves were compared using two-way multivariate analysis of variance (MANOVA) using the SPSS for Windows computer program. Differences between basal gonadotrophin secretion before stimulation with GnRH in testosterone-treated cell cultures of cynomolgus monkeys compared with controls were analysed using the *t*-test for independent samples (SPSS for Windows).

Results

Monkey pituitary cells were separated by an intensified mechanical method of dispersion. The yield of cells/pituitary for rhesus monkeys was $(4{\cdot}5 \pm 1{\cdot}2) \times 10^6$ cells (mean ± S.E.M., n=6); viability was 92 ± 4% (mean ± S.E.M.). In the cynomolgus monkey pituitary cell culture, $3{\cdot}6 \times 10^6$ cells/pituitary with a viability of 92% were obtained. For rats, $(3{\cdot}2 \pm 0{\cdot}2) \times 10^6$ cells/pituitary (mean ± S.E.M.) with a viability of 96 ± 2% (mean ± S.E.M.) were obtained. Controls for cultures treated with testosterone, inhibin and/or GnRH at the end of one experiment showed no significant decrease in cell count or viability (at the beginning of the experiment $(105{\cdot}2 \pm 10) \times 10^3$ cells/ml and 99 ± 1% viable cells; after 48 h incubation with DMEM and change of medium at 44 h $(101{\cdot}6 \pm 16) \times 10^3$ cells/ml and 98 ± 1% viable cells; after 48 h incubation with inhibin $(103{\cdot}2 \pm 9) \times 10^3$ cells/ml and 97 ± 2% viable cells; after incubation with testosterone and GnRH and one change of medium at 44 h $(98{\cdot}6 \pm 12) \times 10^3$ cells and 97 ± 3% viable cells). Medium from the preincubation phase contained debris in small amounts but no cells, and medium obtained during the experiment contained no debris or cells. FSH levels (triplicate wells in three independent experiments

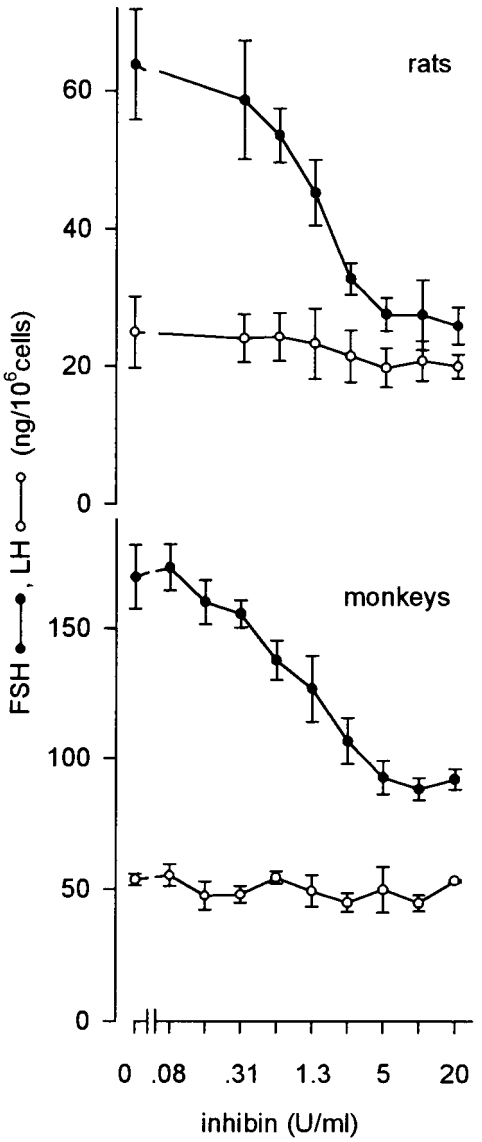

Figure 1 Effect of purified bovine inhibin on the secretion of FSH and LH in pituitary cell cultures of male rats (top) and rhesus monkeys (bottom). The results of three independent experiments (mean ± S.D.) in each species are depicted.

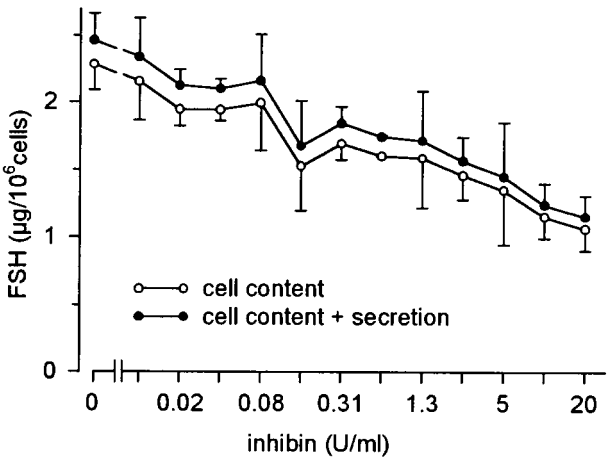

Figure 2 Effect of purified bovine inhibin on cell content of FSH and total FSH (secreted plus cell content) in pituitary cell cultures of five male rhesus monkeys. The results of three independent experiments (mean ± S.D.) with cells from one or two monkeys are depicted.

(n=3 × 3)) ranged from 159·9 to 194·5 ng/10^6 cells for basal secretion and from 76·3 to 103·8 ng/10^6 cells for maximally suppressed secretion during the 48 h incubation.

Effects of inhibin on basal gonadotrophin secretion and cell content in monkeys and rats

Inhibin suppressed FSH secretion in rhesus monkey pituitary cell cultures in a dose-dependent manner to 50·8% (95% range 44·7–56·9) of controls (Fig. 1, bottom). The dose–response curve was of sigmoid shape, and the ED_{50} was 1·024 U/ml (95% range 0·7204 to 1·455). The cell content of FSH and total FSH in the cell culture system at the end of the incubation were likewise decreased (Fig. 2). Levels of inhibin in the serum of normal male rhesus monkeys were determined as described previously (Fingscheidt *et al.* 1989) in eight animals and were 2·8 (0·7–4·9) U/ml (mean, 95% range). Cell cultures of cynomolgus monkey pituitary cells showed comparable secretion of FSH with similar suppression (data not shown). No significant effect of inhibin on LH secretion in rhesus monkey pituitary cells could be detected (P>0·05; ANOVA, followed by Tukey's test; Fig. 1, bottom). We observed no difference between the effect of inhibin on FSH and LH in monkey cell cultures and that in rat pituitary cell cultures (Fig. 1, top). In rats, FSH secretion was suppressed to 45·0% (95% range 40·3–49·6) of controls. The ED_{50} was 1·298 U/ml (95% range 1·064 to 1·584). Inhibin in the serum of male rats was determined in 49 rats in previous studies (Fingscheidt *et al.* 1990, Chandolia *et al.* 1991) and found to be 1·0 (0·7–1·2) U/ml (mean, 95% range).

Effects of testosterone on basal and GnRH-stimulated secretion of FSH and LH in rhesus monkeys

Testosterone at a concentration of 10^{-7} M had no significant effect on basal secretion of FSH or LH during the second preincubation phase of 44 h (Fig. 3), which followed the initial preincubation of 48 h (P>0·05, t-test). GnRH in graded doses, added during the final 4 h of this experiment, stimulated secretion of FSH to maximally 332·7%, and LH to 991·3% of basal secretion. Testosterone at a concentration of 10^{-7} M had no significant effect on GnRH-stimulated gonadotrophin secretion (P>0·05,

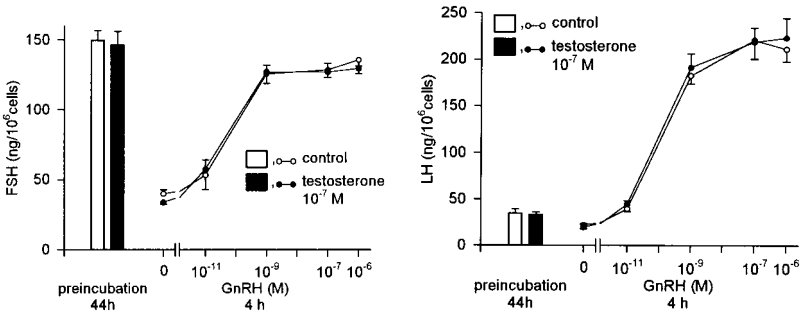

Figure 3 Effect of testosterone (solid bars) at a concentration of 10^{-7} M on basal secretion of FSH (left) and LH (right) in rhesus monkey pituitary cell cultures during the preincubation period of 44 h compared with controls (open bars). After the preincubation phase, the medium was changed and GnRH was added. The curves on the right represent the effect of testosterone (10^{-7} M, ●) on GnRH-stimulated release of FSH (left) and LH (right) during the following 4 h of incubation compared with controls (○). GnRH was added in graded doses from 0 to 10^{-6} M.

MANOVA). With no interaction, GnRH had a significant effect on FSH and LH ($P<0{\cdot}001$, MANOVA). The ED_{50}, (95% range) for stimulation of FSH by GnRH was $6{\cdot}5 \times 10^{-11}$ ($1{\cdot}7 \times 10^{-11}$–$2{\cdot}5 \times 10^{-10}$) M. The corresponding ED_{50} for stimulation of LH by GnRH was higher: $1{\cdot}6 \times 10^{-10}$ ($7{\cdot}6 \times 10^{-11}$–$3{\cdot}2 \times 10^{-10}$) M. Changing the culture medium after 44 h increased the basal LH-release rate from 3·1 to 21·8 ng/10^6cells per 4 h and FSH secretion rose from 13·6 to 39·7 ng/10^6cells per 4 h.

Discussion

We have established and characterized an *in vitro* cell culture model for studying gonadotrophin secretion by pituitaries of non-human primates. With this model we have demonstrated the effects of GnRH, inhibin and testosterone on isolated primate pituitary cells for the first time. To date, only a few reports have appeared on hormone secretion by the pituitary of non-human primates *in vitro*. They were based on tissue culture (Kohler *et al.* 1968, Nicoll *et al.* 1970) or cell culture (Bethea *et al.* 1988) and studied the secretion of prolactin and growth hormone in monkeys.

In humans, the experimental model of cultured pituitary cells has been used by several authors, but only for characterisation of secretion of thyroid stimulating hormone (Abrahamson *et al.* 1987), growth hormone (Markoff *et al.* 1986) and growth hormone and prolactin (Adams *et al.* 1981). Another model for human tissues is based on organ culture and has been used to study gonadotrophin secretion (Groom *et al.* 1971). Finally, tissue and dispersed cells from human pituitary adenomas have been cultured and used to examine gonadotrophin (Mashiter *et al.* 1981) and growth hormone (Le Dafniet *et al.* 1985) secretion. Tissue and organ culture of normal pituitaries are of limited value for investigating gonadotrophin secretion as uncontrollable hormone release results from central necrosis during long-term culture. Gonadotrophin secretion may also be altered in tissue or cells derived from pituitary adenomas. For these reasons our model of dispersed cells from normal pituitaries is probably the best *in vitro* system for studying gonadotrophin secretion.

We have been able to show for the first time that inhibin suppresses FSH secretion by pituitary cells of primates *in vitro*. The effect is the same as that in rat pituitary cells, as demonstrated in our study by pharmacological data from fitted dose–response curves. The ED_{50} values for suppression of FSH by inhibin *in vitro* were within the 95% range for inhibin in the serum of normal rhesus monkeys and rats as determined in previous studies (Fingscheidt *et al.* 1989, 1990, Chandolia *et al.* 1991). The dose–response curves for inhibin were of sigmoid shape, with the steepest part of the curve within the normal range of serum concentrations. Normal secretion of FSH may therefore be under constant suppression by inhibin. However, it must be taken into account that the RIA used for determination of inhibin in the serum of male monkeys reacts not only with the 31 kDa form of inhibin, which was used in the *in vitro* experiments, but also with 59 kDa inhibin and the α-subunit precursor protein pro-α C. In our *in vitro* system, inhibin is a potent regulator of FSH at the pituitary.

Previous *in vitro* studies on the mode of action of inhibin at the pituitary were based on rat (Farnworth *et al.* 1988) and sheep (Clarke *et al.* 1993) pituitary cell cultures. In contrast with the rat, Muttukrishna & Knight (1991) described a stimulating action of inhibin on LH in sheep, whereas the effects on FSH were similar in these two species with a greater sensitivity of the sheep pituitary cells to inhibin (Tsonis *et al.* 1986). The similarity of the effects of inhibin on FSH and LH as characterized by our rat and monkey pituitary cell culture systems underline the value

of the rat model for understanding the physiology of the primate pituitary.

Previous *in vivo* experiments in male rhesus monkeys were based on a preparation known as the hypophysiotropic clamp. Hypothalami of these animals were lesioned and replaced with an invariant intermittent GnRH pump, thus eliminating components of the feedback system acting at the hypothalamic site. In this setting, immunoneutralization of inhibin led to selective hypersecretion of FSH with no effect on LH (Medhamurthy *et al.* 1990). Inhibin affected only FSH in rats, but had no effect on basal secretion of LH. This result is in contrast with a previous finding that both gonadotrophins were suppressed by inhibin in rats *in vitro* (Farnworth *et al.* 1988). This difference between two similar static culture systems in different laboratories remains unexplained. In dynamic *in vitro* models of superfused cells, however, a suppressive effect of inhibin could only be demonstrated for GnRH-stimulated secretion of LH, but not for basal release of LH (Kotsuji *et al.* 1988). These data support our results, since we only examined basal gonadotrophin release for effects of inhibin. In monkey pituitary cells, LH was not influenced by inhibin either, which is comparable with our data for rats.

Since only a limited number of pituitary cells was available for experiments, we decided to study dose–responses for two hormones, GnRH and inhibin. Testosterone was only applied in one dose, known to be maximally effective in rat pituitary cell cultures (Kitahara *et al.* 1991). The concentration chosen for testosterone is equivalent to double the upper normal range in normal male monkeys (Weinbauer *et al.* 1986, Winters *et al.* 1992). In contrast with the reported stimulating action of testosterone on FSH in rat pituitary cell cultures (Kitahara *et al.* 1991, Winters *et al.* 1992), we found no effect of testosterone at the dose of 10^{-7} M on basal or GnRH-stimulated secretion of FSH in monkeys. *In vivo* data in monkeys provide increasing evidence for the view that testosterone has no effect on FSH at the pituitary (Dubey *et al.* 1987, Abeyawardene & Plant 1989, Medhamurthy *et al.* 1991, Majumdar *et al.* 1995) and that inhibin is the testicular hormone responsible for regulation of FSH at the pituitary site.

No significant effect of testosterone on either basal or GnRH-stimulated release of LH could be demonstrated in our monkey pituitary cell culture system. This result parallels the observations of others on rat pituitary cell cultures (Gharib *et al.* 1990, Winters *et al.* 1992), although some authors were able to demonstrate an inhibitory effect of testosterone on GnRH-stimulated LH release in rats (Liang *et al.* 1984). Again, *in vivo* data for monkeys support our *in vitro* data. After castration, only a slight increase in LH was seen in monkeys with a hypophysiotropic clamp, instead of the dramatic postcastration hypersecretion of LH in animals with intact hypothalami (Plant & Dubey 1984). Adams *et al.* (1988) demonstrated, in contrast with our findings, that testosterone increases GnRH-stimulated LH release in prepubertal male monkeys. They suggested that the pituitary might be a target for the feedback action of testosterone on LH. Winters *et al.* (1992) found contradictory results when measuring production of mRNA for subunits of gonadotrophins. Both suppressing and stimulating effects of testosterone were found. In a more recent study, GnRH-stimulated FSHβ mRNA levels were found to be suppressed by testosterone in mouse pituitary cell cultures and transgenic hypogonadal mice (Kumar & Low 1995).

The hypothalamus is supposed to be one site for the feedback action of testosterone in human males (Kerrigan *et al.* 1994). However, an additional site of action of testosterone at the pituitary was proposed by Finkelstein *et al.* (1991) and by Sheckter *et al.* (1989). These *in vivo* studies are limited by a possible effect of testosterone on remaining endogenous GnRH and do not represent an ideal model for studying effects at the pituitary level.

The dose–response characteristics of the action of GnRH on the release of gonadotrophins in our monkey pituitary cell culture system revealed that LH and FSH were stimulated by GnRH in the same manner, with equal mean effective doses, but the extent to which secretion could be stimulated beyond basal values differed greatly. LH could be stimulated tenfold whereas FSH was only stimulated threefold. In addition, a supply of fresh medium after 44 h increased the secretion rate for LH sevenfold compared with threefold for FSH. The *in vitro* secretion of FSH appears to occur more independently of culture conditions and GnRH stimulation than that of LH. This underlines the relative importance of inhibin as the major feedback hormone for FSH.

We conclude that the pituitary is the target organ for inhibin in selectively controlling FSH in non-human primates. Comparing dose–response curves for inhibin with the inhibin concentration in the serum of monkeys suggests that the physiological secretion of FSH may be constantly suppressed by inhibin. Testosterone had no effect in our *in vitro* system and therefore the feedback mechanism of this steroid may be mediated exclusively via the hypothalamus.

Acknowledgements

The authors thank Professor D M de Kretser (Institute of Reproduction and Development, Monash University, Clayton, Victoria, Australia) and Dr D M Robertson (Prince Henry's Institute of Medical Research, Monash Medical Centre, Clayton, Victoria, Australia) for supplying purified inhibin. We are most thankful to R Sandhowe, M Heuermann and G Stelke for their excellent technical assistance. Parts of this work were presented at the Second Pituitary Congress, Palm Springs, CA, USA in 1989. The study was supported in part by the Deutsche

Forschungsgemeinschaft (DFG) (grants Fi 465/1–1 and Ni 130/11–1 (A1)).

References

Abeyawardene SA & Plant TM 1989 Bilateral orchidectomy and concomitant testosterone replacement in the juvenile male rhesus monkey (*Macaca mulatta*) receiving an invariant intravenous gonadotropin-releasing hormone (GnRH) infusion results, as in the hypothalamus lesioned GnRH-driven adult male, in a selective hypersecretion of follicle-stimulating hormone. *Endocrinology* **125** 257–259.

Abrahamson MJ, Wormald PJ & Millar RP 1987 Neuroendocrine regulation of thyrotropin release in cultured human pituitary cells. *Journal of Clinical Endocrinology and Metabolism* **65** 1159–1163.

Adams EF, Brajkovich IE & Mashiter K 1981 Growth hormone and prolactin secretion by dispersed cell cultures of a normal human pituitary: effects of thyrotrophin releasing hormone, theophylline, somatostatin, and 2-bromo-alpha-ergocryptine. *Acta Endocrinologica* **98** 345–351.

Adams LA, Clifton DK, Bremner WJ & Steiner RA 1988 Testosterone modulates the differential release of luteinizing hormone and follicle-stimulating hormone that occurs in response to changing gonadotropin-releasing hormone pulse frequency in the male monkey, *Macaca fascicularis*. *Biology of Reproduction* **38** 156–162.

Bagatell CJ, Dahl KD & Bremner WJ 1994 The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. *Journal of Andrology* **15** 15–21.

Bethea CL, Sprangers SA, West NB & Brenner RM 1988 The effect of simultaneous versus sequential estradiol and progesterone treatments on prolactin production in monkey pituitary cell cultures. *Endocrinology* **122** 1786–1800.

Chandolia RK, Weinbauer GF, Fingscheidt U, Bartlett JM & Nieschlag E 1991 Effects of flutamide on testicular involution induced by an antagonist of gonadotrophin-releasing hormone and on stimulation of spermatogenesis by follicle-stimulating hormone in rats. *Journal of Reproduction and Fertility* **93** 313–323.

Clarke IJ, Rao A, Fallest PC & Shupnik MA 1993 Transcription rate of the follicle stimulating hormone (FSH) beta subunit gene is reduced by inhibin in sheep but this does not fully explain the decrease in mRNA. *Molecular and Cellular Endocrinology* **91** 211–216.

Dubey AK, Zeleznik AJ & Plant TM 1987 In the rhesus monkey (*Macaca mulatta*), the negative feedback regulation of follicle-stimulating hormone secretion by an action of testicular hormone directly at the level of the anterior pituitary gland cannot be accounted for by either testosterone or estradiol. *Endocrinology* **121** 2229–2237.

Farnworth PG, Robertson DM, de Kretser DM & Burger HG 1988 Effects of 31 kilodalton bovine inhibin on follicle-stimulating hormone and luteinizing hormone in rat pituitary cells *in vitro*: actions under basal conditions. *Endocrinology* **122** 207–213.

Fingscheidt U, Weinbauer GF, Robertson DM, de Kretser DM & Nieschlag E 1989 Radioimmunoassay of inhibin in the serum of male monkeys. *Journal of Endocrinology* **122** 477–483.

Fingscheidt U, Weinbauer GF, Khan SA & Nieschlag E 1990 Follicle-stimulating hormone stimulates inhibin in the serum of male monkeys (*Macaca mulatta*). *Acta Endocrinologica* **122** 96–100.

Finkelstein JS, O'Dea LS, Whitcomb RW & Crowley WF Jr 1991 Sex steroid control of gonadotropin secretion in the human male. I. Effects of testosterone administration in normal and gonadotropin-releasing hormone-deficient men. *Journal of Clinical Endocrinology and Metabolism* **73** 621–628.

Forage RG, Ring JM, Brown RW, McInerney BV, Cobon GS, Gregson RP, Robertson DM, Morgan FJ, Hearn MTW, Findlay JK, Wettenhall REH, Burger HG & de Kretser DM 1986 Cloning and sequence analysis of cDNA species coding for the two subunits of inhibin from bovine follicular fluid. *Proceedings of the National Academy of Sciences of the USA* **83** 3091–3095.

Gharib SD, Leung PC, Carroll RS & Chin WW 1990 Androgens positively regulate follicle-stimulating hormone beta-subunit mRNA levels in rat pituitary cells. *Molecular Endocrinology* **4** 1620–1626.

Groom GV, Groom MA, Cooke ID & Boyns AR 1971 The secretion of immuno-reactive luteinizing hormone and follicle-stimulating hormone by the human foetal pituitary in organ culture. *Journal of Endocrinology* **49** 335–344.

Hyde CL, Childs G, Wahl LM, Naor Z & Catt KJ 1982 Preparation of gonadotroph-enriched cell populations from adult rat anterior pituitary cells by centrifugal elutriation. *Endocrinology* **111** 1421–1423.

Kamel F, Balz JA, Kubajak CL & Schneider VA 1987 Gonadal steroids modulate pulsatile luteinizing hormone secretion by perifused rat anterior pituitary cells. *Endocrinology* **120** 1651–1657.

Kerrigan JR, Veldhuis JD & Rogol AD 1994 Androgen-receptor blockade enhances pulsatile luteinizing hormone production in late pubertal males: evidence for a hypothalamic site of physiologic androgen feedback action. *Pediatric Research* **35** 102–106.

Khan SA & Diczfalusy E 1983 Heterologous radioimmunoassay for monkey gonadotrophins. Asessment of the reagents proposed for the assay of FSH. *Acta Endocrinologica* **104** 15–22.

Kitahara S, Kotsuji F, Keeping HS, Oshima H, Troen P & Winters SJ 1991 Interrelationship between the actions of testosterone and primate Sertoli cell inhibin in the control of gonadotropin secretion by cultured pituitary cells. *Endocrinology* **128** 710–716.

Kohler PO, Bridson WE & Rayford PL 1968 Cortisol stimulation of growth hormone production by monkey adenohypophysis in tissue culture. *Biochemical and Biophysical Research Communications* **33** 834–840.

Kotsuji F, Winters SJ, Keeping HS, Attardi B, Oshima H & Troen P 1988 Effects of inhibin from primate Sertoli cells on follicle-stimulating hormone and luteinizing hormone release by perifused rat pituitary cells. *Endocrinology* **122** 2796–2802.

Kreysing P & Nieschlag E 1987 Direct chemiluminescence assay for testosterone. In *Bioluminescence and Chemiluminescence. New Perspectives,* pp 269–272. Eds J Schölmerich, R Andreesen, A Kap, M Ernst & WG Wood. Chichester: Wiley and Sons.

Kumar TR & Low MJ 1995 Hormonal regulation of human follicle-stimulating hormone-beta subunit gene expression: GnRH stimulation and GnRH-independent androgen inhibition. *Neuroendocrinology* **61** 628–637.

Kurshid S, Weinbauer GF & Nieschlag E 1991 Effects of administration of testosterone and gonadotrophin-releasing hormone (GnRH) antagonist on basal and GnRH-stimulated gonadotrophin secretion in orchidectomized monkeys. *Journal of Endocrinology* **129** 363–370.

Le Dafniet M, Garnier P, Brandi AM, Bression D, Scherrer H, Racadot J & Peillon F 1985 Interaction between somatostatin and TRH on growth hormone secretion in perifused human growth hormone tumor cells. *Hormone Research* **21** 235–239.

Liang T, Brady EJ, Cheung A & Saperstein R 1984 Inhibition of luteinizing hormone (LH)-releasing hormone-induced secretion of LH in rat anterior pituitary cell culture by testosterone without conversion to 5 alpha-dihydrotestosterone. *Endocrinology* **115** 2311–2317.

Majumdar SS, Mikuma N, Ishwad PC, Winters SJ, Attardi BJ, Perera AD & Plant TM 1995 Replacement with recombinant human inhibin immediately after orchidectomy in the hypophysiotropically clamped male rhesus monkey (*Macaca mulatta*) maintains follicle-stimulating hormone (FSH) secretion and FSH beta messenger ribonucleic acid levels at precastration values. *Endocrinology* **136** 1969–1977.

Markoff E, Lee DW, Culler FL, Jones KL & Lewis UJ 1986 Release of the 22 000- and the 20 000-dalton variants of growth hormone *in vivo* and *in vitro* by human anterior pituitary cells. *Journal of Clinical Endocrinology and Metabolism* **62** 664–669.

Mashiter K, Adams E & Van Noorden S 1981 Secretion of LH, FSH, and PRL shown by cell culture and immunocytochemistry of human functionless pituitary adenomas. *Clinical Endocrinology* **15** 103–112.

Mason AJ, Hayflick JS, Ling N, Esch J, Ueno N, Ying SY, Guillemin R, Niall H & Seeburg PH 1985*a* Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor-β. *Nature* **318** 659–663.

Mason AJ, Niall HD & Seeburg PH 1985*b* Structure of two human ovarian inhibins. *Biochemical and Biophysical Research Communications* **135** 957–964.

Mayo KE, Cerelli GM, Spiess J, Rivier J, Rosenfeld MG, Evans RM & Vale W 1986 Inhibin A-subunit cDNAs from porcine ovary and human placenta. *Proceedings of the National Academy of Sciences of the USA* **83** 5849–5853.

Medhamurthy R, Abeyawardene SA, Culler MD, Negro Vilar A & Plant TM 1990 Immunoneutralization of circulating inhibin in the hypophysiotropically clamped male rhesus monkey (*Macaca mulatta*) results in a selective hypersecretion of follicle-stimulating hormone. *Endocrinology* **126** 2116–2124.

Medhamurthy R, Culler MD, Gay VL, Negro Vilar A & Plant TM 1991 Evidence that inhibin plays a major role in the regulation of follicle-stimulating hormone secretion in the fully adult male rhesus monkey (*Macaca mulatta*). *Endocrinology* **129** 389–395.

Mercer JE, Clements JA, Funder JW & Clarke IJ 1987 Rapid and specific lowering of pituitary FSH beta mRNA levels by inhibin. *Molecular and Cellular Endocrinology* **53** 251–254.

Muttukrishna S & Knight PG 1991 Inverse effects of activin and inhibin on the synthesis and secretion of FSH and LH by ovine pituitary cells *in vitro*. *Journal of Molecular Endocrinology* **6** 171–178.

Nicoll CS, Parsons JA, Fiorindo RP, Nichols CW Jr & Sakuma M 1970 Evidence of independent secretion of prolactin and growth hormone *in vitro* by adenohypophyses of rhesus monkeys. *Journal of Clinical Endocrinology and Metabolism* **30** 512–519.

Plant TM & Dubey AK 1984 Evidence from the rhesus monkey (*Macaca mulatta*) for the view that negative feedback control of luteinizing hormone secretion by the testis is mediated by a deceleration of hypothalamic gonadotropin-releasing hormone pulse frequency. *Endocrinology* **115** 2145–2153.

Rivier C, Corrigan A & Vale W 1991 Effect of recombinant human inhibin on gonadotropin secretion by the male rat. *Endocrinology* **129** 2155–2159.

Robertson DM, deVos FL, Foulds LM, McLachlan RI, Burger HG, Morgan FJ, Hearn MT & deKretser DM 1986 Isolation of a 31 kDa form of inhibin from bovine follicular fluid. *Molecular and Cellular Endocrinology* **44** 271–277.

Scott RS, Burger HG & Quigg H 1980 A simple and rapid *in vitro* bioassay for inhibin. *Endocrinology* **107** 1536–1542.

Sheckter CB, Matsumoto AM & Bremner WJ 1989 Testosterone administration inhibits gonadotropin secretion by an effect directly on the human pituitary. *Journal of Clinical Endocrinology and Metabolism* **68** 397–401.

Solano AR, Dufau L & Catt KJ 1979 Bioassay and radioimmunoassay of serum luteinizing hormone in the male rat. *Endocrinology* **105** 372–381.

Stouffer RL, Dahl KD, Hess DL, Woodruff TK, Mather JP & Molskness TA 1994 Systemic and intraluteal infusion of inhibin A or activin A in rhesus monkeys during the luteal phase of the menstrual cycle. *Biology of Reproduction* **50** 888–895.

Tilbrook AJ, de Kretser DM & Clarke IJ 1993 Human recombinant inhibin A and testosterone act directly at the pituitary to suppress plasma concentrations of FSH in castrated rams. *Journal of Endocrinology* **138** 181–189.

Tsonis CG, McNeilly AS & Baird DT 1986 Measurement of exogenous and endogenous inhibin in sheep serum using a new and extremely sensitive bioassay for inhibin based on inhibition of ovine pituitary FSH secretion *in vitro*. *Journal of Endocrinology* **110** 341–352.

Weinbauer GF, Surmann FJ, Akhtar FB, Shah GV, Vickery BH & Nieschlag E 1984 Reversible inhibition of testicular function by a gonadotropin hormone-releasing hormone antagonist in monkeys (*Macaca fascicularis*). *Fertility and Sterility* **42** 906–914.

Weinbauer GF, Marshall GR & Nieschlag E 1986 New injectable testosterone ester maintains serum testosterone of castrated monkeys in the normal range for four months. *Acta Endocrinologica* **113** 128–132.

Winters SJ, Ishizaka K, Kitahara S, Troen P & Attardi B 1992 Effects of testosterone on gonadotropin subunit messenger ribonucleic acids in the presence or absence of gonadotropin-releasing hormone. *Endocrinology* **130** 726–734.

Received 29 January 1998
Accepted 26 May 1998