CLINICAL STUDY

FSH secretion predominates *in vivo* and *in vitro* in patients with non-functioning pituitary adenomas

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Abstract

Objective: Non-functioning pituitary adenomas (NFPAs) are characterised by the lack of symptoms of hormone hypersecretory syndromes but *in vitro* studies have demonstrated that tumour cells may stain for gonadotrophins and/or their α - or β -subunits. In this study, we aimed to examine the pattern of secretion of LH and FSH from a series of pituitary adenomas cultured *in vitro* and where data were available to relate the results to pre-operative serum gonadotrophin levels.

Methods: The *in vitro* secretion of LH and FSH was measured from 46 cultured NFPAs and compared with pre-operative serum gonadotrophin levels in 38 patients. Peri-tumorous 'normal' pituitary cell cultures from 20 additional pituitary tumour patients were used for comparison with the NFPA group. **Results:** A median pre-operative LH:FSH ratio of 0.33:1 was found in 38 patients with NFPAs. Preferential secretion of FSH was also documented from media of 46 NFPAs cultured *in vitro* with a median LH:FSH ratio of 0.32:1. A significant correlation ($r = 0.43$, $P < 0.01$) was observed between serum and media levels of FSH but not LH. Peri-tumorous 'normal' pituitary cells released LH and FSH in a reversed ratio (median LH:FSH ratio = 3.6:1, $P < 0.01$ compared with NFPAs).

Conclusions: This study has evaluated pre-operative serum gonadotrophin levels and *in vitro* release of hormones in cultures of surgically removed tissue from patients with NFPAs. The data suggest preferential secretion of FSH occurs both *in vitro* and *in vivo*. By demonstrating that NFPAs cultured *in vitro* reflect the *in vivo* situation of preferential secretion of FSH, it may be possible in future to perform functional studies using this system to elucidate the cellular and molecular mechanisms involved in the development of an imbalance in gonadotroph cells preferentially overproducing FSH in NFPAs.

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Introduction

The prevalence of clinically non-functioning pituitary adenomas (NFPAs) is variously reported as between 15 and 45% of human pituitary tumours. NFPAs are characterised by the lack of symptoms of hormone hypersecretory syndromes and typically present with signs of mass effect when the tumour has reached the stage of a macroadenoma. *In vitro* studies of NFPA tissue have demonstrated that tumour cells may stain for gonadotrophins and/or their α - or β -subunits and usually express transcription factors associated with gonadotroph differentiation such as steroidogenic factor-1 and/or dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome, gene 1 (DAX1) (1–5). These observations have supported the concept that 80–90% of NFPAs are derived from proliferation of gonadotroph cells and led to their proposed reclassification as gonadotrophinomas (6). *In vitro* studies have also shown that a significant proportion of NFPAs synthesise and secrete luteinising hormone (LH) and follicle-stimulating hormone (FSH) or their α -/ β -subunits (1–5). In one-third of such

tumours the quantity of FSH β -subunit mRNA is reported to be in excess of the level of α -subunit mRNA (7). This is not observed in normal pituitary cells where α -subunit production predominates and assembly of the β -subunit is regarded as the rate-limiting step in hormone production. Whereas evidence for increased gonadotrophin synthesis is commonly seen *in vitro*, excess hormone production is much less commonly seen *in vivo*. Serum levels of FSH have been reported as increased in up to 15% of patients with NFPAs (1), although the percentage of FSH secretors may in reality be higher since detection can be complicated in postmenopausal women. However, a recent study comparing gonadotrophin levels in healthy subjects with NFPA patients, all aged > 50 years, showed levels of gonadotrophins in female patients which were far lower than controls, and in male patients a large overlap of gonadotrophin values with controls was observed (8). This is most likely due to the late stage of presentation of these patients such that the tumour mass compresses the normal pituitary, thereby impairing hormone production. LH-secreting adenomas are rarely seen (9) and an elevated ratio of

α -subunit to LH and FSH found *in vivo* in women with NFPA probably results directly from α -subunit over-secretion by tumour cells rather than hypopituitarism secondary to tumour bulk (10). There are a number of possible explanations for the observed discrepancy between *in vitro* and *in vivo* results. LH and FSH are secreted in a pulsatile manner *in vivo* and this pattern is altered in subjects with NFPA. In contrast to the synchronous pattern of release of LH and FSH seen in normal subjects, pulses of FSH from subjects with gonadotrophinomas are of increased frequency and display an erratic pattern and loss of synchrony with LH pulses (11). A further diagnostic difficulty may lie in the heterogeneity of the FSH molecule itself, with variations in glycosylation leading to alterations in circulating half-life (12). This has complicated the investigation into the contribution of changes in levels of hormone release as opposed to changes in half-life on circulating hormone levels. The pre-operative identification of gonadotrophinomas remains a diagnostic difficulty, and currently immunohistochemical staining is required for the postoperative diagnosis of this tumour phenotype. The aetiology of gonadotrophinomas is also unclear and clear identification of increased FSH production would contribute to our understanding of the development of these tumours. In this study we aimed to examine the pattern of secretion of LH and FSH from a large series of pituitary adenoma cells cultured *in vitro* and where data were available to relate the results to pre-operative serum gonadotrophin levels.

Methods

Clinical details and patient selection

Pituitary tumours were collected from patients at the time of transphenoidal adenomectomy. Tissues were divided at the time of surgery for diagnostic histological studies and for tissue culture. All subjects gave informed consent, at the time of operation, for surgical specimens to be used for diagnostic and research purposes and the study was approved by the Local Ethics Committee. Patients who presented due to mass effect, without clinical or hormonal features of pituitary hormone secretion, were classified as clinically NFPA. After surgery, all tumours were examined for morphological and immunocytochemical characteristics by light microscopy. Patients with a macroadenoma and a serum prolactin (PRL) <2000 mU/l were not excluded from the NFPA group unless the surgically removed tissue immunostained positively for PRL. Tumours were excluded from the NFPA series if microscopy or immunostaining suggested an alternative histological diagnosis, if significant normal pituitary tissue was present on microscopy, or if the tissue appeared necrotic.

Morphology and immunocytochemistry

All tumours were examined by standard haematoxylin and eosin, reticulin and periodic acid-Schiff stains, and routine immunostaining was performed for growth hormone (GH), PRL, adrenocorticotrophin (ACTH), thyrotrophin (TSH), LH and FSH (antibodies against the whole molecule obtained from BioGenex Laboratories, Inc., Wokingham, Berks, UK), and α -subunit (rabbit polyclonal; UCB Bioproducts, Braine-L'Alleud, Belgium) using a standard streptavidin-biotin-horse-radish peroxidase method. An adenoma was considered positive for a pituitary hormone or α -subunit when more than 10% of cells reacted with specific antibody. The distinction between normal pituitary gland and tumour was confirmed by reticulin staining in all cases.

Assays for pituitary hormones

GH, PRL, LH, FSH and TSH were measured using two-site chemiluminescent enzyme immunoassays on an Immulite auto-analyser (Euro/DPC Ltd, Llanberis, Gwynedd, UK). The intra- and inter-assay coefficients of variation for all of these assays are less than 4 and 8% respectively. ACTH was measured by a specific double antibody RIA (Euro/DPC Ltd) with inter- and intra-assay coefficients of variation of less than 10%. α -Subunit concentrations were measured by a direct double antibody RIA using antibodies purchased from UCB Bioproducts and chloramine-T-iodinated antigen (National Institute for Biological Standards and Control reagent 76/508; Potters Bar, Herts, UK) and were calibrated against the First International Reference Preparation 75/569 (National Institute for Biological Standards and Controls). Intra- and inter-assay coefficients of variation were less than 6 and 11% respectively. Cross-reactivities (nanograms per nanogram) with purified LH, FSH and TSH were 3.6, 1.9 and 1.3% respectively. The detection limits of the pituitary hormone assays, defined as the concentration 2 s.d. above the response at zero dose, were as follows: GH, 0.5 mU/l; PRL, 10 mU/l; LH, 0.3 IU/l; FSH, 0.3 IU/l; TSH, 0.008 mU/l; ACTH, 4 pmol/l; α -subunit, 0.1 μ g/l. All samples from each individual tumour were analysed in the same assay. Hormone data were initially obtained as concentrations, but were then corrected for cell number and incubation time. The data presented are therefore expressed as the amount secreted per 10^6 cells/24 h. The reported detection limits following normalisation to cell number and incubation time were as follows: GH, 2.0 μ U; PRL, 50 μ U; LH, 0.5 mIU; FSH, 0.5 mIU; TSH, 0.1 μ U; ACTH, 20 fmol; α -subunit, 0.5 ng.

Statistical analysis

All statistical analysis was performed using GraphPad Prism software (GraphPad Software, Inc., San Diego,

CA, USA). Data on *in vitro* hormone secretion did not conform to a normal distribution, and were not amenable to transformation. Non-parametric analyses were therefore used throughout. Spearman rank correlation coefficients (*r*) were calculated to examine the correlations between media or serum levels of each hormone and the Mann–Whitney U-test was used for comparison of LH:FSH ratios and median levels of gonadotrophins between groups. For all tests, $P < 0.05$ was considered statistically significant.

Results

Overview of study patients

NFPAs were collected from 82 subjects who presented with mass effects or hypopituitarism but without clinical evidence of hormone hypersecretion. The distinction between a gonadotrophin-secreting adenoma and an NFPA was not attempted pre-operatively. Our studies were based on those tumours where the histological specimen was composed of adenoma tissue and light microscopy excluded significant normal pituitary. Following this examination, 19 tumours were excluded from the series leaving 63 adenomas in the study. The 63 adenomas were sub-classified as LH/FSH adenomas ($n = 22$) when immunocytochemistry reported that more than 10% of the cells reacted with specific antibodies to LH and/or FSH, or null cell adenomas ($n = 41$) when no such staining was observed. These null cell adenomas also failed to demonstrate staining for GH, PRL or TSH. Three of the null cell adenomas stained positively for ACTH and five null cell adenomas were positive for α -subunit. Pre-operative serum LH and FSH levels were available for 38 out of the 63 patients. These 38 tumours comprised 13 LH/FSH adenomas and 25 null cell adenomas. Table 1 demonstrates the correlation between the immunocytochemical expression of LH and/or FSH and the *in vitro*

secretion of LH and/or FSH in the 38 adenomas where pre-operative serum LH and FSH levels were available. The Table shows that 11 out of 13 LH/FSH adenomas and 10 out of 25 null cell adenomas also expressed LH and/or FSH *in vitro*. The other two LH/FSH adenomas secreted α -subunit *in vitro*, as did seven out of 25 null cell adenomas. For the *in vitro* secretion analyses we used the 11 LH/FSH adenomas and 10 null cell adenomas documented in Table 1, together with the additional 25 NFPAs (nine LH/FSH adenomas and 16 null cell adenomas) where pre-operative serum LH and FSH levels had not been available.

We were keen to contrast *in vitro* secretion data from NFPAs with normal human pituitary. As a surrogate for normal tissue, we analysed the *in vitro* secretion in a series of 33 patients where transphenoidal surgery had been undertaken for the presence of hormone-secreting pituitary adenomas and a significant amount of normal pituitary tissue was present on histological examination. In 13 of these 33 cases, the cultured cells predominantly released hormones expected from the original diagnosis and were excluded. However, in 20 cases (five from patients diagnosed pre-operatively with acromegaly, 10 with Cushing's disease, four with presumed PRLomas and one with evidence of a TSHoma) basal hormone secretion measured *in vitro* contained hormones from all cell types. It is likely that this pattern represents the secretion of peri-tumorous 'normal' pituitary tissue and data from culture of these tumours were used for comparison with the NFPA group.

FSH secretion predominates *in vivo*

Pre-operative serum gonadotrophin levels were available for 38 (18 males, 20 females) patients with NFPAs (Table 2). The patients ranged in age from 16 to 81 years (median = 66 years old). LH levels were in the low normal range or decreased in all patients except

Table 1 Comparison of immunocytochemistry (ICC) and *in vitro* results in the NFPAs ($n = 38$) in which pre-operative serum gonadotrophin levels were available.

Adenoma type	Positive for LH/FSH by ICC	Secreting LH/FSH <i>in vitro</i>	Secreting α -subunit alone
LH/FSH adenomas ($n = 13$)	100% (13/13)	85% (11/13)	15% (2/13)
Null cell adenomas ($n = 25$)	0% (0/25)	40% (10/25)	28% (7/25)

Table 2 Serum FSH and LH levels in patients with NFPAs.

	<i>n</i>	Median age (years (range))	Median LH (IU/l (range))	Median FSH (IU/l (range))
Males	18	66 (16–81)	2.0 (0.3–15.7)	4.6 (0.3–279)
Normal ranges	—	—	1–10	1–8
Females	20	32 (30–33)	2.8 (0.7–4.8)	7.9 (3.8–11.9)
Premenopausal	2	—	2.5–70	1–25
Normal ranges	—	—	—	—
Postmenopausal	18	67 (48–80)	3.3 (0.3–28.6)	12.0 (1.2–53.5)
Normal ranges	—	—	> 16.0	> 25.0

one male and two postmenopausal females. FSH levels were elevated in six male patients (66%). Twelve postmenopausal females (66%) had levels of FSH that were lower than the normal postmenopausal range. Only two of the six postmenopausal females with FSH levels in the normal postmenopausal range also had LH levels comparable to those observed in normal females. FSH was more frequently elevated than LH with a median LH:FSH ratio of 0.33:1 (range = 0.001–1.26). This group of patients comprised 13 LH/FSH adenomas and 25 null cell adenomas (see Table 1) and we were therefore interested to compare the serum LH:FSH ratios between these groups. The median LH:FSH ratio was 0.29:1 (range = 0.001–1.00) in the LH/FSH adenoma group and 0.40:1 in the null cell adenoma group. There was no significant difference in the LH:FSH ratio between these groups ($P = 0.19$, ns). The increased release of FSH (median = 7.5 IU/l, range <0.3–279 IU/l) compared with LH (median = 2.3 IU/l, range <0.3–28.6 IU/l) amongst these patients is illustrated in Fig. 1. Although a significant correlation between serum LH and FSH was observed in this group (Spearman $r = 0.62$, $P < 0.001$), all but three points in the scattergram fell above the line of equality (dotted line). Figure 2 demonstrates that there were no significant gender differences between the serum concentrations of LH in men and women (median LH 2.0 IU/l, range <0.3–15.7 in men; median LH 3.5 IU/l, range <0.3–28.6 in women, $P = \text{ns}$), or FSH in men and women (median FSH 4.4 IU/l, range <0.3–279 in men; median FSH 11.5 IU/l, range 1.2–53.5 in women, $P = \text{ns}$).

Figure 1 Correlation between pre-operative serum LH and FSH levels in 38 subjects with NFPAs. Spearman rank ($r = 0.62$, $P < 0.001$). The line of equality is drawn as a dotted line and demonstrates that in all but three patients FSH was greater than LH.

Figure 2 Scattergrams illustrating pre-operative serum LH and FSH levels in 18 male (M) and 20 female (F) subjects with NFPAs. There were no significant gender differences between serum LH and FSH concentrations of men and women (Mann–Whitney U-test, P value not significant).

NFPAs: *in vitro* results

As detailed above, 46 adenomas (20 LH/FSH adenomas and 26 null cell adenomas) secreted measurable amounts of LH and FSH *in vitro*. Figure 3 illustrates the correlation between LH and FSH levels in media from these cultured tumours and shows a reduction in the preferential secretion of FSH observed *in vivo* with the data points being scattered about the line of equality. However, as with the *in vivo* data, a significant correlation between *in vitro*-secreted LH and FSH was observed (Spearman $r = 0.40$, $P < 0.01$) and interestingly, in these tumours the median LH:FSH ratio was 0.32:1 (range = 0.01–41.3), an almost identical ratio to that observed *in vivo*. Again, there was no

Figure 3 Correlation between LH and FSH levels in media of cultured NFPAs ($n = 46$). Spearman rank ($r = 0.40$, $P < 0.01$). Each data point represents the amount of LH and FSH (mIU) per 10^6 cells per 24 h secreted by each adenoma. The line of equality is drawn as a dotted line.

significant difference in the median LH:FSH ratio between the LH/FSH adenoma group (median LH:FSH ratio = 1.05:1, range = 0.002–41.33, $n = 20$) and the null cell adenoma group (median LH:FSH ratio = 0.27:1, range = 0.009–6.67, $n = 26$, $P = \text{ns}$ compared with the LH/FSH adenoma group).

Serum FSH correlates with FSH secretion *in vitro*

In order to determine whether the pattern of hormone secretion observed *in vitro* corresponded to *in vivo* levels, *in vitro* gonadotrophin measurements were compared with the pre-operative serum gonadotrophin levels in the 38 subjects where these data were available. Scattergrams showing the relationship between *in vitro* and *in vivo* LH and FSH secretion for each subject are shown in Fig. 4A and B. No significant correlation was evident between *in vitro* LH secretion and serum LH (Spearman $r = 0.09$, $P = \text{ns}$, Fig. 4A). However, a significant correlation was noted between serum FSH and *in vitro* FSH secretion ($r = 0.43$, $P < 0.01$, Fig. 4B), suggesting that the measured pre-operative serum FSH is indeed coming from adenomatous tissue. Taken together, the above data indicate that NFPAs preferentially secrete FSH *in vivo*. Although they maintain the capacity to release LH, this becomes more apparent *in vitro* and suggests that *in vivo* LH may have been under inhibitory control.

In vitro gonadotrophin secretion by 'normal' human pituitary

To determine whether the reduction of FSH predominance *in vitro* was a consequence of the dispersal and culture procedure and removal of circulating inhibitory regulators of LH, we compared the secretion pattern of the NFPAs in this series to peri-tumorous 'normal' pituitary tissue removed from 20 subjects during transphenoidal exploration as described above. LH and FSH were released in a reversed ratio (median LH:FSH = 3.6:1, range = 0.4–22.7, $n = 20$) to that seen in the NFPA group but still with a highly significant correlation between LH and FSH ($r = 0.76$, $P < 0.001$, Fig. 5). Compared with the data from NFPAs the median LH:FSH ratio was considerably greater than 1.0 with most points falling below the line of equality. This relationship is markedly different from that seen in NFPAs (compare with Fig. 3) where the median ratio of LH to FSH was considerably less than 1.0. Comparison of the *in vitro* LH and FSH ratios between NFPAs (median = 0.32:1, range = 0.01–41.3) and normal pituitary (median = 3.6:1, range = 0.4–22.7) is shown in Fig. 6. These data indicate again that in the majority of peri-tumorous 'normal' pituitary specimens, LH is released in preference to FSH. In NFPA tumour specimens, although a wide variation was observed in the LH:FSH ratio, the median LH:FSH ratio was significantly

Figure 4 Correlation between LH (A) and FSH (B) levels in media of cultured NFPAs and pre-operative serum LH (A) and FSH (B) concentrations. Values in media are expressed per 10^6 cells per 24 h. No correlation was evident for LH (Spearman rank ($r = 0.09$, $P = \text{ns}$, $n = 38$) but a significant correlation was demonstrated for FSH (Spearman rank ($r = 0.43$, $P < 0.05$, $n = 38$).

lower ($P < 0.01$) than that from the peri-tumorous 'normal' tissue.

Discussion

In this study we have examined gonadotrophin levels *in vivo* and *in vitro* in a series of NFPAs. Our data suggest imbalanced secretion of gonadotrophins in favour of FSH in serum samples collected pre-operatively from

Figure 5 Correlation between LH and FSH levels in media of cultured tumours from other patients with a clinical diagnosis of hormone-secreting adenoma ($n = 20$). Each data point represents the amount of LH and FSH per 10^6 cells per 24 h secreted by each adenoma. Spearman rank ($r = 0.76$, $P < 0.001$). The line of equality is drawn as a dotted line and demonstrates that in all but two adenomas LH secretion was greater than FSH.

Figure 6 Comparison of LH:FSH ratios in media of cultured NFPAs ($n = 46$) or tumours from other patients with a clinical diagnosis of hormone-secreting adenoma ($n = 20$). The median value for each group is shown as a horizontal line. Mann–Whitney U-test, $*P < 0.01$.

these patients. In addition, we have documented preferential secretion of FSH from cultured cells of these adenomas, although secretion of LH was also apparent *in vitro*. The significant correlation observed between levels of serum and media FSH but not LH, together with identical LH:FSH ratios, suggests that the culture system is reflecting the *in vivo* situation. It is now widely accepted that NFPAs are derived from the proliferation of cells of the gonadotroph lineage which synthesise LH or FSH and/or their α - and β -subunits, but rarely does this synthetic capacity result in elevated serum gonadotrophin levels. In an early study of 22

patients with NFPAs, only five out of 22 subjects (23%), all of whom were men, had hypersecretion of serum FSH or LH β - or α -subunit (10). Although these authors did not report LH:FSH ratios, examination of their published data shows that 21 out of 22 patients had LH:FSH ratios < 1.0 . Other studies have reported similarly low incidences (4–17%) of elevated gonadotrophins in patients with NFPAs (2, 13–16). Our data are similar in that six out of 38 patients (16%), again all of whom were men, had hypersecretion of FSH. Similarly, 35 out of 38 patients had LH:FSH ratios < 1.0 while the remaining three patients had values very close to 1.0. Interestingly, when we sub-classified the group into LH/FSH adenomas or null cell adenomas based on immunocytochemical staining, there was no significant difference in the serum LH:FSH ratios between the groups, suggesting that the preferential secretion of FSH *in vivo* occurs irrespective of immunocytochemical findings in surgically removed tissue. Takeda *et al.* (17) found exclusively higher FSH:LH ratios (i.e. lower LH:FSH ratios) in a subset of five FSHomas, compared with other pituitary adenomas, including NFPAs, PRLomas and GHomas. Following pituitary surgery, the FSH:LH ratio decreased in the FSHomas and became similar to that found in the other tumour types. However, the criterion used by these authors to distinguish between FSHomas and NFPAs (absence of immunohistochemical staining for FSH β -subunit) raises some questions about the cellular origins of the tumours in their NFPA group. In contrast to the predicted gonadotrophin hypersecretion, a more frequent finding in patients with NFPAs are low levels of LH and FSH compared with an age-matched healthy population. A recent study of serum gonadotrophin levels in 47 patients (aged 50–80 years) with NFPAs (8) reported that female patients showed levels of gonadotrophins which were far lower than those observed in age-matched healthy women. In male patients, serum FSH levels were similar to those in age-matched controls, although patients did show a significant reduction in LH when compared with healthy men, again supporting a decrease in the LH:FSH ratio. Our results of low normal or decreased LH levels in all 38 patients, except for one male subject and two postmenopausal females, support these findings. Few studies have evaluated pre-operative serum gonadotrophin levels and *in vitro* release of hormones in cultures of surgically removed tissue from the same patients. Kwekkeboom *et al.* (10) reported no correlation between the amounts of LH and FSH released *in vitro* and the serum hormone values in 20 patients with NFPAs, but LH and FSH were released in only seven and nine cultures respectively of the 20 tumours studied, making correlations difficult to interpret. We were able to compare data from 38 subjects and found no correlation for LH but did demonstrate a significant correlation for FSH, as has been shown

previously by Wessels *et al.* (18) in a small study of patients with gonadotrophinomas ($n = 4$) and NFPAs ($n = 14$). This positive correlation between serum and media FSH suggests that the cultures are reflecting the *in vivo* situation as far as FSH secretion is involved. The documentation of LH release *in vitro* despite low levels of LH *in vivo* and the lack of correlation between serum and *in vitro* LH implies the presence of LH-specific inhibitory factors *in vivo*. It is tempting to speculate that this might relate to circulating levels of testosterone or oestradiol but although almost all pituitary tumours express both oestrogen receptor- α and - β (19), oestrogen-induced negative feedback is reported as disrupted in the majority of patients with gonadotrophinomas and in half of those with NFPAs (20). Thus the explanation for low serum LH levels in patients with NFPAs remains unclear. However, despite the appearance of LH secretion *in vitro*, our finding of identical LH:FSH ratios *in vitro* and *in vivo* would suggest that FSH release also increased proportionately *in vitro*. The availability of resected normal human pituitary for culture studies is very limited. While accepting the limitations of our data from peri-tumorous 'normal' cells, it was interesting to observe the dramatic reversal in LH:FSH ratio seen in these samples. This again emphasises the dominance of FSH secretion from NFPA samples. Recent publications have focused on the roles played by activin, inhibin, follistatin and bone morphogenetic proteins (BMPs) in FSH overproduction. At the pituitary level, activin acts in a local manner to stimulate FSH expression and secretion and has an antiproliferative effect, with these actions being blocked by follistatin and inhibin (21). A positive correlation has been demonstrated in NFPAs between activin and FSH by two groups (17, 18). Other studies have shown that the antiproliferative effect of activin may be lost in some gonadotrophinomas (22). This is thought to be due to the expression of truncated receptor isoforms of the main Type 1 activin receptor (Alk4) in a small proportion of gonadotrophinomas, which act in a dominant negative way to interfere with wild-type receptor function and block the action of activin (23). If this is the case then it is difficult to see how activin signalling could also be involved in preferential production of FSH. Takeda *et al.* (17) report a reduction in follistatin mRNA in gonadotrophinomas compared with NFPAs and suggest that the inhibitory action of follistatin is thus lost in gonadotrophinomas allowing unopposed action of activin and BMPs on FSH synthesis and secretion. Our study would suggest that the majority of NFPAs demonstrate preferential FSH production, irrespectively of subsequent classification by immunocytochemistry into LH/FSH adenomas and null cell adenomas and thus a clear distinction between gonadotrophinomas and NFPAs as reported by others is seldom possible. Our data would thus support the proposed reclassification of NFPAs as gonadotrophinomas as suggested by Chaidarun & Klibanski

(6). By demonstrating that cultured cells from NFPAs continue to reflect the *in vivo* situation of preferential secretion of FSH, it may be possible in future to perform functional studies using this system to elucidate the cellular and molecular mechanisms involved in the development of an imbalance in gonadotroph cells preferentially overproducing FSH in NFPAs.

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