Effects of long-term treatment with the gonadotropin-releasing hormone analog nafarelin in patients with non-functioning pituitary adenomas

Paolo Colombo, Bruno Ambrosi, Katia Saccomanno, Monique Bassetti, Donatella Cortelazzi and Giovanni Faglia

Institute of Endocrine Sciences; Department of Pharmacology, CNR Center of Cytopharmacology, University of Milan, Milan, Italy


The supposed origin of non-functioning pituitary adenomas (NFPA) from gonadotrophs prompted us to investigate the effects of the gonadotropin-releasing hormone (GnRH) analog nafarelin on hormonal and tumoral parameters in eight patients with NFPA, previously unsuccessfully operated and all hypogonadal. Nafarelin was administered intranasally for 1 year to all patients. Four patients received a dose of 1200 μg/day; the remaining four received 800 μg/day for 3 months, which was subsequently increased to 1200 μg/day. Basal gonadotropin and α-subunit (αSU) levels were low–normal. In four patients (nos. 1, 2, 3, 5) nafarelin significantly lowered luteinizing hormone (LH) levels, and also follicle-stimulating hormone (FSH) in three of them (nos. 1, 2, 3). Persistent FSH stimulation occurred in three patients (nos. 6, 7, 8), with a transient slight LH increase only in patient no. 8. In one patient (no. 7), αSU levels were persistently stimulated. Hormonal responses to an acute GnRH test during nafarelin administration were generally blunted when compared to the pretreatment responses. Immunofluorescence results, obtained before treatment in five adenomas (nos. 2, 3, 4, 6, 7), had been as follows: positive for FSH-β in all; negative for LH-β in all, except a few positive cells in case no. 4; positive for αSU in three (nos. 2, 3, 7). No changes of visual field and tumor size occurred in any patient during treatment. However, one patient who showed a persistent increase in FSH levels exhibited left palpebral ptosis after 12 months of therapy and underwent a second transphenoidal surgery. In conclusion: NFPA behave heterogeneously in terms of hormonal responses to GnRH analog therapy; long-term nafarelin treatment was unsuccessful in reducing the size of NFPA; and stimulation rather than inhibition of gonadotropin levels may suggest discontinuance of GnRH analog therapy in NFPA.

Paolo Colombo, Istituto di Scienze Endocrine, Ospedale Maggiore IRCCS-Pad, Granelli, via F. Sforza 35, 20122 Milano, Italy

Non-functioning pituitary adenomas (NFPA) represent approximately 25% of all pituitary tumors and are not associated with clinical signs and symptoms of hormone overproduction. However, the term “non-functioning” is suitable only from a clinical point of view. Increasing evidence is provided by a number of in vitro studies that many of these adenomas can secrete (1–9), positively stain (1, 2, 7–10) and express genes (8, 10) for intact gonadotropins and/or their free subunits. Moreover, they can present functioning receptors for gonadotropin-releasing hormone (GnRH), because release of gonadotropins (3, 7), increased inositol phospholipid turnover (11) and intracellular free calcium mobilization (12) have been reported to occur in vitro in these tumors following GnRH administration. The hypothesis of their origin from gonadotroph cells has been suggested (13).

Non-functioning pituitary adenomas are often large tumors when diagnosed, as patients do not show the classic clinical pictures caused by hormone hypersecretion and come later to clinical observation. As a consequence, surgery and radiant therapy are often partially effective. The attempt of reducing the mass of these adenomas by means of medical therapy is currently under investigation. In this respect, the presence and secretion of gonadotropins and their free subunits by NFPA, along with their sensitivity to GnRH, has raised the possibility that these tumors could respond to GnRH agonists, which are known to inhibit gonadotropin secretion in normal subjects when chronically administered (14).

Up to now, the administration of various GnRH agonists (buserelin, goserelin, leuprolide, tryptorelin) was reported to cause tumor mass shrinkage in only a minority of patients with NFPA or true gonadotropinomas, with variable changes of circulating LH, FSH and α-subunit (αSU) levels (15–21).
In this work, we report on the effects of long-term intranasal administration of d-Nal(2)\(^+\)-GnRH (nafarelin), a GnRH analog that is 200 times more potent than native GnRH (22), on hormonal and tumoral parameters in eight patients with NFPA.

Subjects and methods

Eight patients—five females (nos. 1,2,3,6,7) and three males (nos. 4,5,8)—aged 58–78 years—with NFPA were studied, who had undergone previous incomplete adenomectomy by transphenoidal surgery (TSS), followed by conventional external radiant therapy (RT) in four of them (nos. 1,5,6,8). Morphological and immunofluorescence investigations of the excised adenomas had been carried out in five patients (nos. 2,3,4,6,7). Based on electron microscopy, three patients (nos. 2,3,6) bore null cell adenomas, while in two (nos. 4,7) the diagnosis was of oncocytoma. Immunofluorescence studies (Table 1) showed positive immuno-reactivity for FSH-\(\beta\) in all five patients; it was intense and present in most adenomatous cells of patient nos. 2,3 and 7, but weak and occurring only in 10% and 5% of cells in case nos. 4 and 6, respectively. No specific immunoreaction for LH-\(\beta\) was obtained, except for a few positive cells (4%) in the tumor of patient no. 4. In three patients (nos. 2,3,7), and particularly in patient no. 2, the adenoma showed an intense fluorescence for \(\alpha\)SU in most of the observed cells, while \(\alpha\)SU immunoreactivity was negative in patient nos. 4 and 6. Moreover, in cultured cells obtained from the excised adenoma from patient nos. 2 and 4, the administration of GnRH (100 nmol/l) into culture medium caused a rise of intracellular free calcium, as described previously (12).

At the moment of inclusion in the present study (0.5–18.0 and 1.5–18.0 years after TSS and RT, respectively), a high-resolution computed tomography (CT) scan or magnetic resonance imaging (MRI) showed a tumoral residue confined within the pituitary fossa in five patients (nos. 1,2,3,4,7), a microadenoma with marked suprasellar extension in patient no. 6 and adenomas with slight suprasellar extension in case nos. 5 and 8. Five patients (nos. 3,4,6,7,8) were on substitutive therapy with cortisone (37.5 mg/day po) and \(\alpha\)thyroxine (100–150 µg/day po). Monthly parenteral administration of testosterone esters was discontinued in the three males in order to avoid interference at the hypothalamic–pituitary level.

Intrasenal nafarelin (Syntex Research, UK) administration was started and continued for 1 year in all patients. In four patients (nos. 2,3,4,5) the drug was administered at the dose of 1200 µg/day t.i.d., while patient nos. 1,6,7 and 8 received a dose of 800 µg/day b.i.d. for the first 3 months, which subsequently was increased to 1200 µg/day t.i.d.

Before treatment, and then at monthly intervals, blood samples were taken for the evaluation of LH, FSH, \(\alpha\)SU, estradiol-17\(\beta\) (E\(_2\)) and total testosterone, except for patient no. 3 in whom blood samples were taken every 3 months.

The GnRH test (100 µg iv), CT scan or MRI of the sellar region and visual field determinations were performed before and at the 6th and 12th month of therapy in all subjects, except for patient no. 6 who did not undergo the GnRH test at the 12th month.

Hormonal assays

Circulating levels of LH and FSH were measured by ultrasensitive time-resolved IFMA assays (Delfia\textsuperscript{®} hLH and hFSH kits, Pharmacia SpA, Milan, Italy). The intra- and interassay coefficients of variation were, respectively, 4.5% and 9.5% for LH and 5.2% and 7.9% for FSH. The sensitivity of the IFMA was 0.03–0.07 U/l for LH and 0.02–0.06 U/l for FSH. Standard curves were prepared using the following International Reference Preparations (WHO): 80/552 for LH and 78/549 for FSH. The addition of increasing amounts of TSH, FSH and \(\alpha\)SU (up to \(10^{-8}\) mol/l) to 2 \(\times\) \(10^{-10}\) mol/l (40 U/l) LH did not cause any variation in the assay counts, suggesting that these molecules do not interfere or cross-react in LH IFMA measurement. Similarly, LH, TSH and \(\alpha\)SU did not cross-react in the FSH IFMA.

Circulating levels of \(\alpha\)SU were measured by previously described methods (23). The assay sensitivity was 0.1–0.3 µg/l and the intra- and interassay coefficients of variation were below 7.8% and 9.6%, respectively.

Serum testosterone and E\(_2\) levels were measured in unextracted serum by specific direct RIA methods (Coat-A-Count, Diagnostic Products Corporation, USA, and Biodata SpA, Italy, respectively). The intra- and interassay coefficients of variation were 6.3% and 10.4% for testosterone and 7.5% and 9.0% for E\(_2\), respectively. The lower limit of sensitivity of the assays was 0.1 nmol/l for both hormones.

Normal basal values were considered as follows: in postmenopausal women, LH 20–100 U/l, FSH 20–120 U/l, E\(_2\) < 0.1 nmol/l, \(\alpha\)SU 0.8–4.2 µg/l; in males, LH 0.2–6.5 U/l, FSH 0.5–8.0 U/l, testosterone 13–42 nmol/l, \(\alpha\)SU 0.1–0.9 µg/l.

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*Table 1. Immunofluorescence results in five non-functioning pituitary adenomas.*

<table>
<thead>
<tr>
<th>Patient</th>
<th>LH-(\beta)</th>
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<th>(\alpha)-Subunit</th>
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<td>++</td>
</tr>
<tr>
<td>No. 3</td>
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<td>±</td>
<td>–</td>
</tr>
<tr>
<td>No. 7</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Fluorescence intensity: –, negative; ±, weak; +, strong; ++, very strong.*
Results

Hormonal results

Patient nos. 1, 2 and 3. In these three female patients basal gonadotropin levels were low or subnormal for age, except for normal FSH levels in patient no. 2. Nafarelin administration induced a reduction of LH and FSH levels that persisted throughout the whole period of therapy. Circulating basal αSU levels were normal in patient nos. 1 and 2 and low in patient no. 3. They remained unchanged in patient nos. 1 and 3, while in patient no. 2 an irregular pattern was observed, with two peaks gradually attained at the 2nd and 7th month of therapy, each followed by a return of αSU into the basal range (Fig. 1). The rise of LH, FSH and αSU levels after an acute GnRH test was significant in patient nos. 1 and 2 and slight in patient no. 3 before therapy. During nafarelin administration, the LH response was absent and the FSH response was lower in all cases, except for patient no. 3 at the 6th month, while the response of αSU was reduced in case nos. 1 and 2 and absent in case no. 3 (Table 2). In each patient, low basal E2 levels (< 0.1 nmol/l), according to postmenopausal age, were unmodified by nafarelin.

Fig. 1. Gonadotropin and α-subunit (αSU) levels before and during nafarelin treatment in three patients with non-functioning pituitary adenomas: patient no. 1 (○); patient no. 2 (●); patient no. 3 (△).

Fig. 2. Gonadotropin and α-subunit (αSU) levels before and during nafarelin treatment in two patients with non-functioning pituitary adenomas: patient no. 4 (○); patient no. 5 (●).
Table 2. Gonadotropin (U/l) and α-subunit (αSU (μg/l)) responses to acute GnRH test in eight patients with non-functioning pituitary adenomas, before and during nafarelin treatment.

<table>
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<td>0.7</td>
</tr>
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<td>—</td>
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<td>—</td>
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<tr>
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<td>0.6</td>
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</tr>
<tr>
<td></td>
<td>αSU</td>
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</table>

Patient nos. 4 and 5. In these two male patients the basal LH levels were in the low−normal range. They were reduced by nafarelin therapy in patient no. 5, but only occasionally in patient no. 4. Normal FSH and αSU basal levels did not change in case no. 4 and showed only a minimal increase in patient no. 5 throughout the whole period of treatment (Fig. 2). The pretreatment gonadotropin and αSU responses to the acute GnRH test were very slight in both patients and were blunted further after 6 and 12 months of therapy (Table 2). A reduction of low basal testosterone levels occurred in both patients (no. 4: from 2.3 to 0.6 ± 0.2 nmol/l; no. 5: from 9.1 to 1.2 ± 0.2 nmol/l; mean ± sem).

Patient nos. 6, 7 and 8. Basal circulating gonadotropin levels were low in patient nos. 6 and 7 and subnormal in patient no. 8. All three patients showed a rise of FSH levels during the first 3 months while taking the 800 μg/day dose of nafarelin. After increasing the dose to 1200 μg/day, the FSH levels were lowered in all patients but always remained higher than the basal levels. A similar trend in LH levels occurred in patient no. 8, but subsequently the LH level returned to within the basal range; on the contrary, in patient nos. 6 and 7 the LH levels were unchanged, except for an occasional slight peak at the 5th month of treatment. Normal or low basal αSU levels were recorded in all three patients. A rise of αSU levels occurred in patient no. 7 throughout the whole period of therapy, while no changes were observed in patient nos. 6 and 8 (Fig. 3).

Before treatment, the GnRH test induced a very slight or no increase of LH, FSH and αSU levels. With the exception of a slightly greater gonadotropin response at the 6th month of therapy in patient no. 8, the responses to the GnRH test remained absent or were blunted further during nafarelin administration (Table 2). In the two female patients, basal E₂ levels were in the postmenopausal range (E₂ < 0.1 nmol/l) and were unmodified by nafarelin therapy. In patient no. 8, low basal testosterone levels (4.7 nmol/l) fell to undetectable levels.

Ophthalmological and radiological results

In all patients no changes of visual field or tumoral mass size occurred at CT scan or MRI of the sellar region after 6 and 12 months of therapy with nafarelin. In spite of these findings, patient no. 6 experienced left palpebral ptosis at the 12th month of treatment and underwent a second transphenoidal surgery.

Discussion

The results of immunofluorescence investigations that had been obtained in five tumors confirm the general observation that NFPA frequently synthesize intact
gonadotropins or their free subunits, recent works have demonstrated that the amount of hormone release by these adenomas is generally low and attributable only to a limited number of tumoral cells (4, 9). Along with damage of normal gonadotroph cells by tumoral compression, previous surgical or radiant therapy, this fact also could explain the finding of subnormal or low basal gonadotropin levels in our patients. None of them had high αSU levels in basal conditions.

In the eight patients studied, the patterns of gonadotropin and αSU responses to long-term intranasal nafarelin administration were rather heterogeneous and similar to those reported by various authors during treatment with other GnRH analogs, either in true gonadotropinomas or in NFPA. In some cases a persistent agonist effect on gonadotropin and αSU levels had been observed (16, 18–21), while others showed reduction (17, 19, 21) or no changes (15, 21) of hormonal values.

The doses of nafarelin administered intranasally to our patients were comparable or even higher than those able to induce a reduction of sex hormone levels in normal subjects (23) and in patients with hormone-dependent conditions, such as endometriosis and precocious puberty (24, 25).

The efficacy of these doses was demonstrated in four patients (nos. 1, 2, 3, 5) by a reduction of LH levels throughout the whole period of therapy, accompanied by a lowering of FSH in three of them (nos. 1, 2, 3). However, the results of immunofluorescence obtained in two of them had been negative for LH-β, suggesting at least for LH, an effect of nafarelin on normal gonadotroph cells.

Different considerations can be drawn in the three patients (nos. 6, 7, 8) who showed an elevation of FSH levels during nafarelin treatment. In patient no. 7 the FSH rise was rather marked with the 800 µg/day dose of nafarelin, with a peak attained at the 3rd month. When the dose was augmented to 1200 µg/day, FSH levels were lowered but remained constantly well above baseline levels. Immunofluorescence analysis of the tumor from this patient had resulted in strong FSH-β positivity in most adenomatous cells. The same occurred in patient no. 6, in whom an FSH rise was constantly present, though less marked, with slight FSH-β immunofluorescence scattered in 4% of tumoral cells. Negative LH-β immunofluorescence had been recorded in the tumors of both patients, and they did not show any significant change of LH levels during nafarelin treatment. No immunofluorescence data had been obtained in patient no. 8, who also showed a rise of FSH levels similar to that of patient no. 6. These data might allow us to speculate that in patient nos. 6 and 7 the prolonged administration of nafarelin could have induced a paradoxical substantial release of FSH by the tumor, allowing us to detect higher circulating hormonal levels. This suggestion does not contradict the recent observation that NFPA exhibit poor

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Fig. 3. Gonadotropin and α-subunit (αSU) levels before and during nafarelin treatment in three patients with non-functioning pituitary adenomas: patient no. 6 (○); patient no. 7 (●); patient no. 8 (□).
hormonal secretion in vitro, because these studies were performed either in basal conditions or under acute GnRH stimulation (4, 9). Moreover, a rise of gonadotropin levels during GnRH analog therapy was a common finding in most patients with gonadotropinomas examined so far (16, 18–20).

Regarding the pattern of αSU response during nafarelin therapy, only patient no. 7 exhibited a substantial and persistent increase of the free subunit, while patient no. 2 showed two αSU peaks each followed by a return to the basal range. Both patients had a strong positive αSU immunofluorescence in the tumor specimen. In the first case it was accompanied by a rise of FSH levels, and in the latter by suppression of gonadotropin levels. The increase of αSU, recorded in most patients with gonadotropinoma, also was shown to occur in normal humans, concomitantly with suppression of gonadotropin levels (26, 27). For this reason its attribution to an increased αSU release by the tumor seems rather difficult.

Variable patterns of gonadotropin and αSU responses to an acute GnRH test during nafarelin administration were recorded. Although these responses were generally blunted if compared to the pretreatment responses, the complete unresponsiveness of all three parameters was achieved only occasionally during the whole period of treatment, suggesting that intranasal nafarelin did not exert a constant and complete inhibition of gonadotropin and αSU release to an acute GnRH test.

The results of neuroradiological investigations, performed during nafarelin treatment in order to evaluate the possible effect of the drug on tumor mass, were disappointing. In fact, a reduction of tumor size was not observed at CT scan or MRI in any of the eight patients studied after 6 and 12 months of therapy.

Out of all the studies carried out with GnRH analogs on true gonadotropinomas, a partial tumor shrinkage was observed only in one patient, reported by Zarate et al. (17), while in NFPA this occurred in 2/11 patients, reported by Liuzzi et al. (21). In this respect, our work confirms that GnRH analog therapy is unsuccessful in reducing the size of most of these tumors. At present, however, no definite conclusions on the efficacy of this form of treatment can be drawn. Apart from the need for larger series of patients, it would be of great importance to characterize the receptorial status of the adenoma in order to select those patients who potentially could benefit from this form of treatment; the lack of these studies could explain, at least in part, the low success rate with GnRH analog therapy observed so far in these tumors.

Moreover, the possibility of clinical worsening due to enlargement of the adenoma during GnRH analog administration also should be considered. In one of our patients (no. 6) this was suggested because, in spite of unchanged visual field and CT scan imaging of the sellar region, left palpebral ptosis occurred after 12 months of therapy and the patient needed a second transsphenoidal surgery. Although the possibility of tumoral growth independent of nafarelin action cannot be excluded, it should be noted that in this patient a sustained elevation of FSH levels occurred throughout the whole period of nafarelin therapy. In this respect, it would not be inconceivable that the long-term and persistent agonist effect induced by nafarelin, probably on tumoral hormone secretion, may have played a role in inducing tumor growth rather than shrinkage in this patient. This fact could be of relevance, because a paradoxical increase of gonadotropin levels during GnRH analog therapy was not an uncommon finding in previous series of patients with gonadotropinomas. On the contrary, an inhibition of gonadotropin levels during GnRH analog therapy was accompanied by the shrinkage of a true gonadotropinoma, reported by Zarate et al. (17), while the pattern of hormonal response is not known in the two patients with NFPA who showed tumor size reduction, reported by Liuzzi et al. (21). The increase in size of an NFPA, causing progressive diplopia and headache, has been reported also to occur only 7 days after a single intramuscular administration of a depot tryptorelin formulation (28). Therefore, GnRH analogs potentially are not devoid of serious adverse effects strictly related to the presence of the pituitary adenoma; the establishment of this form of therapy, in order to try to reduce the size of non-functioning or gonadotropin-secreting pituitary adenomas, should imply careful clinical, biochemical and neuroradiological follow-up. It might be possible also that the use of recently available GnRH antagonists, acting as receptor blockers rather than receptor downregulation, will allow better results. Effective suppression of FSH levels in patients with FSH-secreting gonadotropinomas has been reported with the GnRH antagonist Nal-Glu GnRH (20, 29); in one of these patients this had not been achieved with the GnRH agonist leuprolide (20). However, McGrath et al. (29) did not obtain any reduction of tumor size in five gonadotropinomas treated with Nal-Glu GnRH for 3–12 months.

In conclusion:
(i) different gonadotropin and αSU responses were obtained in eight patients with NFPA treated with the GnRH agonist nafarelin, intranasally, for 1 year, confirming that these adenomas behave heterogeneously to this form of treatment;
(ii) intranasal nafarelin administration did not reduce tumor size in any of the eight patients, further supporting the view that GnRH analog therapy is not an effective medical treatment in NFPA;
(iii) stimulation rather than inhibition of gonadotropin levels during GnRH analog therapy might predict enlargement of tumor size and suggest that therapy be discontinued.

References
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