Multihormonal response to corticotropin-releasing hormone in inferior petrosal sinus blood of one patient with Cushing’s disease: comparison with in vitro secretion of the tumoral corticotropes

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A multihormonal response to CRH during inferior petrosal sinus sampling in patients with Cushing’s disease has recently been described. Whether it reflects multihormonal secretion by the corticotropic adenoma, or secretion by non-tumorous adjacent cells via paracrine mechanisms remains debatable. We have compared the effect of CRH on ACTH, GH, PRL and TSH secretion during inferior petrosal sinus sampling with its effect on the in vitro secretion of the corticotropic adenoma after excision in one case of Cushing’s disease. Before CRH injection in vivo results show significant central–peripheral gradients for all hormones but only ACTH lateralized to the side of the tumor. After CRH administration, the petrosal concentrations of all hormones increased preferentially on the side of the adenoma resulting in significant intersinus gradients: 8.1 for ACTH, 2.0 for GH, 1.8 for PRL and 1.5 for TSH. In vitro results: the adenoma cells were immunostainable for ACTH only. In culture, they secreted ACTH only. Addition of CRH to the culture induced a mean increase of 160% in ACTH secretion but GH, PRL and TSH remained undetectable. Our results favor the hypothesis that the multihormonal response to CRH seen during inferior petrosal sinus sampling in Cushing’s disease reflects a paracrine stimulation of the adjacent non-tumorous pituitary cells by the corticotropic adenoma.

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Bilateral and simultaneous inferior petrosal sinus (IPS) sampling offers a new opportunity for the assessment of pituitary secretion, as it allows the measurement of hormones in relatively undiluted blood draining from the pituitary. Comparison of ACTH concentration between inferior petrosal sinus and peripheral blood has proven to be useful for the diagnosis of Cushing’s disease (1, 2). The combination of IPS sampling with corticotropin-releasing hormone (CRH) injection amplifies the ACTH gradient between the petrosal and peripheral levels and therefore increases the diagnostic power of the procedure (2, 3). Some investigators have also claimed that IPS sampling can be used to predict the localization of corticotroph adenomas to one side of the pituitary (2–4). Recently, other pituitary hormones such as GH, PRL and TSH have been reported to be increased in the IPS on the side of lateralized adenoma in patients with Cushing’s disease (5–11). Intriguingly, the concentration of these hormones increased after CRH injection (7, 9–11). The mechanisms underlying these observations remain unknown. A multihormonal secretion by the corticotropic adenoma has been proposed (6, 7, 10) but this has not definitively been demonstrated and other data support a paracrine action of the tumoral corticotrophs (9, 11).

We have studied the secretion of the whole anterior pituitary gland in vivo in one case of Cushing’s disease using IPS sampling combined with a CRH injection, and compared it with the in vitro secretion of the cultured adenomatous corticotropes after excision. Our results favor the hypothesis that secretory products of the corticotropic adenoma stimulate adjacent normal pituitary cells in a paracrine fashion.

Case report and methods

Case report

A 29-year-old Caucasian female was referred to us presenting typical clinical features of Cushing’s syndrome. It was confirmed by the finding of an increased urinary free cortisol and elevated plasmatic cortisol level (Table 1). The diagnosis of Cushing’s disease was based on the finding of measurable plasma ACTH (Table 1), a fall of plasma cortisol and ACTH averaging 80% and 88% respectively after a high dose of dexamethasone (8
mg/d for 2 d) and the comparison between IPS and peripheral plasma ACTH concentrations (see section results). High resolution angiocomputed tomography scanning of the pituitary revealed a microadenoma of 6 mm in diameter on the left side. The pituitary adenoma was subsequently removed by transsphenoidal pituitary microsurgery. The totality of the tumor was processed for cell culture and no fragment was available for conventional histopathological examination. Surgery was followed by a prolonged isolated ACTH deficiency (Table 1).

### Methods

**IPS sampling.** Bilateral and selective catheterization of the IPS was performed as previously described (3). Briefly, the catheters were inserted through a femoral vein and location of tips was determined before and after the procedure by injection of a contrast medium. Blood was simultaneously collected from both IPS and from a peripheral vein before, 5 and 15 min after iv administration of 100 μg of ovine CRH (UCB Bioproducts, Braine-L’alleud, Belgium). Peripheral blood was also sampled 30 and 60 min after CRH injection. Samples were collected in EDTA tubes, which were kept at 4°C during the procedure, and centrifuged after the last sample. The plasma was kept frozen at −20°C until assayed for ACTH, GH, PRL and TSH. Plasma cortisol concentration was also measured in peripheral blood samples.

The significance of central to peripheral and intersinus gradients was defined as > 1.7 and ≥ 1.4 respectively (3, 4).

### Assays

ACTH was measured by IRMA with a detection limit of 1.5 pmol/l and an intra-assay coefficient of variation (CV) < 6.0% (Allegro HS-ACTH, Nichols Institute, San Juan Capistrano, CA). GH was measured by IRMA with a detection limit of 0.2 μg/l and an intra-assay CV < 4.0% (Elsa-hGH, CIS Bio-International, Gisy-sur-Yvette, France). PRL was measured by IRMA with a detection limit of 1.5 μg/l and an intra-assay CV < 7.0% (Immunootech SA, Marseille, France). TSH was measured by IRMA with a detection limit of 0.03 mU/l and an intra-assay CV < 4.0% (Immunootech SA, Marseille, France). No pituitary hormones were detectable in culture medium unexposed to the cells.

### Results

**IPS sampling results (see Table 2)**

Bilateral IPS catheterization was successfully performed. No serious side effects developed. Before CRH injection, there was a significant central-peripheral gradient for all the hormones studied. It was greater on the tumor-bearing side of the pituitary and reached 12.6 for ACTH, 9.0 for GH, 8.3 for PRL and 1.8 for TSH. The intersinus gradient was significant for ACTH only (1.9 vs 1.1 for GH and PRL, 1.3 for TSH).

After CRH injection, there was an increase of plasma ACTH concentrations at both IPS and peripheral levels.
The increase in IPS was greater than the increase in the periphery resulting in a maximal central-peripheral gradient of 461. The increase in IPS was also clearly greater on the adenoma-bearing pituitary side than on the contralateral side, resulting in an increase of the ACTH inter-sinus gradient from 1.9 to 8.1. The ACTH response was accompanied by a 69% increase in peripheral cortisol concentration.

After CRH injection, GH, PRL and TSH concentrations also increased in the IPS by 119%, 107% and 67% respectively, whereas peripheral concentrations remained unchanged. In other terms, CRH induced central-peripheral gradients of 28.5 for GH, 20.1 for PRL, and 5.0 for TSH. The preferential increase of IPS, GH, PRL and TSH concentrations on the tumor-bearing side induced significant inter-sinus gradients for all the hormones studied: 2.0 for GH, 1.8 for PRL and 1.5 for TSH.

**Immunocytochemistry**

All pituitary cells were ACTH positive. No staining was observed for PRL, GH, TSH or for FSH, LH, TRH, VIP and galamin.

**Adenoma cell culture and in vitro CRH stimulation**

Before CRH injection ACTH was clearly detectable in the culture supernatant of the four wells. The mean (±SEM) ACTH concentration on day 7 was 239.3 ± 38.6 pmol.l⁻¹.d⁻¹. On day 5, ACTH concentration increased by 109% and 212% in the two CRH exposed culture wells and decreased by 11% and 2% in the two unexposed wells. Basal GH, PRL and TSH were undetectable and remained so after CRH exposure.

**Discussion**

IPS catheterization provides a sensitive way of investigating pituitary secretion, as it allows the measurement of pituitary hormones prior to their dilution in the general circulation. This is illustrated by the finding of significant central-peripheral gradients for pituitary hormones even in the absence of secreting pituitary tumor (14). Similarly, GH, PRL, TSH and α-subunit have been reported to be increased in IPS blood from patients with Cushing's disease. In most cases the concentrations of these hormones were higher in the IPS blood ipsilateral to the ACTH increase, resulting in multiple inter-sinus hormone gradients (5–11). Unexpectedly, the gradients of these hormones increased with similar pattern to that of ACTH after CRH injection while their peripheral concentrations remained unchanged (7, 9–11).

The mechanisms that underlie these observations remain unknown, but three considerations must be taken into account. First, the negligible inter-sinus gradients for LH and FSH and the lack of influence of CRH injection on their petrosal concentrations do not support a non-specific tumor effect on the secretion of all pituitary hormones (6, 8, 11). Second, the lateralization of the phenomenon for GH, PRL, TSH and α-subunit suggests that the stimulatory effect of CRH takes place at the pituitary level. Third, the lack of effect of CRH on multihormonal inter-sinus gradients in the case of ectopic ACTH syndrome suggests that functional pituitary corticotropes are a prerequisite for the effect of CRH on other pituitary hormones (9). Therefore, multihormonal gradients may reflect either a co-secretion of pituitary hormones by the corticotropic adenoma or paracrine interactions between the tumor and the adjacent non-tumoral pituitary cells.

The lateralization of multihormonal inter-sinus gradients in most cases, and the growing evidence from histological studies that clinically identified uni-secretory pituitary tumors secrete several hormones (15, 16) support the pituitary adenoma co-secretion hypothesis. Although this has been described in some individual cases (17–19), ACTH-secreting pituitary adenomas rarely secrete GH, PRL and TSH (15, 16). Furthermore, in nearly all previously reported cases of multihormonal inter-sinus gradients, histological examination revealed that the tumors were immunoreactive for ACTH only (5, 6, 8–10). Last, the observation of multihormonal response to CRH in IPS blood in patients with glucocorticoid resistance or hormonally inactive pituitary tumors, favors the hypothesis of hormone release by non-tumorous cells via paracrine mechanisms (9). However, validation of this hypothesis requires the demonstration of a lack of effect of CRH on the release of GH, PRL and

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**Table 2.** Plasma ACTH, GH, PRL and TSH concentrations during bilateral inferior petrosal sinus blood sampling combined with CRH injection in one case of Cushing's disease LIPS and RIPS: left and right inferior petrosal sinuses. Time: time of sampling after CRH injection. Normal values: ACTH [2–13] pmol/l, GH < 15 mU/l, PRL < 15 μg/l and TSH [0.3–4.5] mU/l.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Sampling site</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (pmol/l)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>LIPS</td>
<td>113</td>
<td>4151</td>
</tr>
<tr>
<td>RIPS</td>
<td>59</td>
<td>513</td>
</tr>
<tr>
<td>Peripheral</td>
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<td>9</td>
</tr>
<tr>
<td>GH (μg/l)</td>
<td>2.7</td>
<td>5.7</td>
</tr>
<tr>
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<td>59.7</td>
<td>74.9</td>
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<td>6.7</td>
</tr>
<tr>
<td>Peripheral</td>
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<td>0.2</td>
</tr>
<tr>
<td>PRL (μg/l)</td>
<td>65.2</td>
<td>135.0</td>
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<tr>
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<td>74.9</td>
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<tr>
<td>Peripheral</td>
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<tr>
<td>TSH (mU/l)</td>
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<td>1.5</td>
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<tr>
<td>RIPS</td>
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<tr>
<td>Peripheral</td>
<td>1.8</td>
<td>1.5</td>
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</table>
TSH by the cultured tumor cells from patients who showed a multihormonal response to CRH during bilateral IPS sampling.

Our patient had Cushing’s disease related to a corticotrophic pituitary microadenoma on the left side. The baseline ACTH concentration in the left IPS was clearly higher than in the contralateral IPS and in the periphery. This was also the case for GH, PRL, and TSH, although the basal inter-sinus gradient for these hormones was not significant. However, after CRH injection a preferential increase in the ipsilateral IPS occurred and the inter-sinus gradients became significant for all the hormones studied. A similar discrepancy between basal and post-stimulated inter-sinus gradients has been previously described (10, 11). Immunocytochemistry of the adenoma cells was positive for ACTH and negative for GH, PRL and TSH. The cultured tumor cells secreted only ACTH. The addition of CRH to the culture medium stimulated ACTH secretion, but contrary to in vivo findings, did not influence GH, PRL and TSH secretion. This is the first documented case of a discrepancy between the in vivo and in vitro responses to CRH of corticotrophic tumor cells. Nussey et al. have recently reported similar findings concerning the secretion of oxytocin and AVP in two cases of Cushing’s disease: however, the data are incomplete (11). Our patient’s ACTH deficiency following transsphenoidal pituitary microsurgery suggests that the whole adenoma has been excised. Although a loss of tumor fragments during the removal procedure or processing for culture cannot be excluded, we believe that our in vitro results reflect the secretion from the entire tumor. However, as immunocytochemistry was performed after two days of culture, it cannot be definitely excluded that a weak synthesis of GH, PRL or TSH may have been undetectable by staining or assays.

Although no definite conclusions can be reached from a single observation, these data strongly suggest that the multihormonal inter-sinus gradients seen in Cushing's disease reflect paracrine mechanisms rather than co-secretion of hormones by the tumor. It raises the question of which substance mediates this paracrine effect. A role for β-endorphine has been proposed but recent data do not support this concept (9). Attention has recently been focused on the potential paracrine role of VIP (20), TRH (21) and galanin (22, 23). Furthermore, galanin has recently been identified in human corticotrophic adenomas (24). We did not find any immunostaining suggesting the tumoral cells that we studied could release these neuropeptides. This does not exclude a VIP or galanin-mediated paracrine action since ACTH has recently been shown to stimulate their release from perfused rat anterior pituitaries (25).

In conclusion, our data support the hypothesis that the multihormonal response to CRH in IPS blood of patients with Cushing’s disease is the result of a paracrine action of the tumoral corticotropes on the non-tumorous adjacent pituitary cells. Further studies are needed to conclude whether the multihormonal gradients observed after CRH injection in Cushing’s disease are limited to this pathological situation, or if they reflect physiological interactions between pituitary cell types.

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