Inferior petrosal sinus sampling: Evidence of a stimulatory effect of oCRH on GH secretion in Cushing’s Disease

P. Loli, F. Vignati, G. Scialfa, E. Boccardi, V. Branca, M. E. Berselli

Department of Endocrinology and Neuroradiology, Ospedale Niguarda Ca’ Granda, Milano, Italy

Abstract. Preoperative localization of ACTH-secreting microadenomas has been performed in 9 patients with Cushing’s disease by using bilateral and simultaneous venous sampling of the inferior petrosal sinuses. In addition to ACTH and PRL we determined GH levels after oCRH stimulation in order to confirm the possible occurrence of unilateral GH increases, as recently observed by us in one patient. A central-to-peripheral and an intersinus gradient of ACTH concentration was observed in 5 patients examined before and/or after oCRH stimulation. In 7 patients central-to-peripheral and side-to-side PRL gradients were recorded in basal conditions; in 5 of these patients a unilateral oCRH-induced PRL increase was observed. Six out of the 7 patients with unilateral PRL increases also showed an intersinus GH gradient in basal conditions (ratio ≥1.5); in 5 of them a clearcut oCRH-induced GH increase was observed. A peripheral oCRH-induced GH and PRL increase was not observed in any of the patients. The observation of a paradoxical oCRH-induced GH increase in the inferior petrosal sinus with the higher ACTH concentration is of speculative and clinical interest: whether it reflects co-secretion of hormones by the tumour or hormone release by non-tumourous cells via paracrine mechanisms is still to be clarified. The unilateral GH increase could represent an additional signal of the presence and localization of an ACTH-secreting tumour.

The selective, bilateral and simultaneous catheterization of inferior petrosal sinuses for plasma ACTH determination has become an important tool in the differential diagnosis of ACTH-dependent hypercortisolism (1).

According to some investigators, this procedure can also lead to preoperative localization of an ACTH-secreting microadenoma within the pituitary when the tumour is too small to be visualized with current neuroradiological techniques (2).

It has been recently reported that the unilateral increase in ACTH levels that can be recorded in blood from the inferior petrosal sinus ipsilateral to an ACTH-secreting tumour can be paralleled by a similar secretory pattern of hormones other than ACTH (or the cleavage products of adenosomatous POMC secretion) such as TSH, αHCG, prolactin, and growth hormone (3-7).

Whether this unexpected observation reflects co-secretion of hormones by the tumour or hormone release by non-tumourous cells via paracrine mechanisms is still to be clarified.

We recently found that during selective catheterization of the inferior petrosal sinuses, administration of oCRH resulted in elevation of GH levels in blood from the inferior petrosal sinus ipsilateral to an ACTH-secreting tumour in one patient with ACTH-dependent Cushing’s disease (8). We have now examined a larger number of patients.

Patients and Methods

Nine consecutive patients with hypercortisolism (5 females and 4 males, aged 15-67 years) were studied after giving informed consent.

All patients had undergone an extensive evaluation, including low and high-dose dexamethasone suppression tests, insulin tolerance test, and oCRH stimulation test; the results of these tests were consistent with the diagnosis of ACTH-dependent Cushing’s disease.
Two patients had previously undergone unsuccessful pituitary adenomectomy (Table 1, No. 5 and 8).

One patient (Table 1, No. 1), who was taking α-methyldopa for hypertension, had persistently elevated peripheral serum PRL levels (range 0.9-2.2 nmol/l).

Peripheral serum GH levels, normally low in basal conditions, did not increase in any of the patients after stimulation with insulin-induced hypoglycemia (0.15 IU/kg, iv).

None of the patients had evidence of pituitary adenoma at high-resolution, contrast-medium computerized tomographic scans (Somatom 2N head scanner, Siemens) and (in 8 patients) at Nuclear Magnetic Resonance.

Selective catheterization of inferior petrosal sinuses was performed using a bilateral transjugular approach as previously described (7). Simultaneous blood samples were collected from the inferior petrosal sinuses and from peripheral blood both before and 1, 3, 5, 10 min after injection of 100 μg oCRH (UCB, Bioproducts, Brussels, Belgium) into the antecubital vein. In addition, samples were simultaneously taken in jugular veins and in peripheral blood about 15 min after oCRH injection. Blood samples for ACTH, PRL and GH determination were collected in plastic tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinine (Trasylol®) and immediately centrifuged; plasma was frozen at -20°C until assay.

The determination of plasma ACTH was performed by immunoradiometric assay (IRMA) using commercial kits purchased from Nichols (San Juan de Capistrano, CA). In our laboratory, the normal range for ACTH levels is 2.2 to 17.6 pmol/l; the intra- and the inter-assay coefficients of variation are, respectively, 3.8 and 9.8%.

Plasma PRL levels were determined by RIA (Biodata Kit, Rome, Italy). In our laboratory, the normal range for PRL levels is 0.08 to 0.6 nmol/l; the intra- and inter-assay coefficients of variation are 5.2 and 7.4%, respectively.

The determination of plasma GH was performed by RIA using commercial kits purchased from Sorin (Saluggia, Italy); in our laboratory the normal range for GH levels is <233 pmol/l; the intra- and inter-assay coefficients of variation are 7.6 and 11%, respectively.

All samples from individual patients were analysed at the same time.

Four of the patients examined subsequently underwent selective adenomectomy (Table 1, Nos. 1, 2, 3, and 4).

Data were analysed using the Pearson correlation coefficients.

ACTH, PRL and GH concentrations in blood samples collected before and after oCRH administration are reported in Table 1.

In all patients, the plasma ACTH concentration was higher in blood from one or both inferior petrosal sinuses than in peripheral blood, either in basal conditions (6 patients; higher petrosal sinus/peripheral blood ACTH gradient >2.5) or after oCRH stimulation (9 patients; higher petrosal sinus/peripheral blood ACTH gradient >2.0).

In all subjects, the ACTH concentration lateralized to one side of the pituitary, either in basal conditions (7 patients; intersinus gradient ≥3.0) or after oCRH stimulation (9 patients; intersinus gradient >1.7).

In all the patients examined (in addition to the one with known hyperprolactinemia), the peripheral serum PRL levels were slightly elevated during the sampling procedure and did not change after oCRH stimulation.

In 7 subjects (Nos. 2, 4-9), the basal PRL concentration was higher in one inferior petrosal sinus than in the contralateral one (intersinus gradient ≥1.5) or in the peripheral blood (higher petrosal sinus/peripheral blood PRL gradient ≥1.5); in 5 of these subjects (Nos. 2, 4-7), the PRL concentration showed a clearcut unilateral increase after oCRH stimulation.

In 6 (Nos. 2, 4-8) among the 7 patients who showed side-to-side gradients of PRL, the basal GH concentration was higher in one inferior petrosal sinus than in the contralateral one (intersinus gradient ≥1.5) or in the peripheral blood (higher petrosal sinus/peripheral blood GH gradient ≥1.5); in 5 of these subjects (Nos. 2, 4-7), the GH concentration showed a clearcut unilateral increase after oCRH administration. The highest oCRH-stimulated GH values were recorded 3-5 min after oCRH administration. No oCRH-induced changes in GH concentrations were recorded in peripheral blood.

Basal and oCRH-stimulated PRL and GH concentrations lateralized to the same side as basal and/or oCRH-stimulated ACTH levels.

Four patients have so far undergone pituitary microsurgery (Nos. 1-4); in two of them (Nos. 2, 4) both PRL and GH plasma levels, in addition to ACTH, lateralized to one side of the pituitary and showed unilateral increases after oCRH stimulation. In each case a microadenoma was identified intraoperatively and successfully removed. In these 4 patients the side with the higher petrosal sinus/peripheral ACTH (and, when present, also GH

Results

Catheterization of the inferior petrosal sinuses was accomplished in all 9 patients; it was well tolerated and no complications occurred during or after the procedure.
and PRL) gradients agreed with tumour localization within the pituitary at transsphenoidal surgery; it was subsequently shown that these tumours stained positively for ACTH and negatively for PRL and GH at immunohistochemistry.

A significant positive correlation could be established between the intersinus gradients of GH and PRL concentration in basal conditions (\( r = 0.89, p<0.0001 \)) and after oCRH stimulation (\( r = 0.56, p<0.05 \)). There were also significant correlations

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Sample site</th>
<th>GH (pmol/l)</th>
<th>PRL (nmol/l)</th>
<th>ACTH (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>Left</td>
<td>42</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>47</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>42</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>Left</td>
<td>33</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>98</td>
<td>79</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>28</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>Left</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>47</td>
<td>42</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>Left</td>
<td>37</td>
<td>98</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>23</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>19</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Left</td>
<td>33</td>
<td>37</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>56</td>
<td>56</td>
<td>368</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>23</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>Left</td>
<td>158</td>
<td>126</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>28</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>14</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>Left</td>
<td>350</td>
<td>657</td>
<td>769</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>144</td>
<td>149</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>56</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>Left</td>
<td>42</td>
<td>79</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>28</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>28</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>Left</td>
<td>9</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Peripheral</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>
between the intersinus gradients of basal GH concentration and the intersinus gradients of oCRH-stimulated GH peaks ($r = 0.59$, $p < 0.02$), and between the intersinus gradients of basal PRL concentration and the intersinus gradients of oCRH-stimulated PRL peaks ($r = 0.49$, $p < 0.005$).

**Discussion**

The occurrence of a side-to-side gradient of ACTH and PRL in blood from the inferior petrosal sinuses observed in our patients is in agreement with previous reports (4-6). A more intriguing finding of the present report is the occurrence in 5 out of 9 patients with Cushing's disease of a oCRH-induced clearcut increase in GH levels, concurrent with an ipsilateral increase in ACTH and PRL levels, in blood from one inferior petrosal sinus. The finding is of particular interest, considering that in patients with hypercortisolism GH secretion is consistently decreased even after physiological stimuli (9); accordingly, none of the patients of the present series showed consistent increases in peripheral GH levels after stimulation with insulin-induced hypoglycemia. It appears therefore that peripheral hormone levels do not closely reflect the central pituitary hormone milieu.

It can be speculated that the unilateral oCRH-induced GH increase could be related to the presence of an ACTH-secreting tumour in the side of the pituitary ipsilateral to the inferior petrosal sinus with the higher GH, as well as ACTH and PRL concentrations; this possibility is substantiated by several observations. First of all, two among the GH-responding patients actually had surgical and histopathological confirmation of an ACTH-secreting adenoma (the localization of which within the pituitary agreed with the side with higher petrosal sinus/peripheral GH, ACTH and PRL gradients).

Second, a positive correlation was found between both basal and oCRH-stimulated intersinus gradients of GH and PRL, suggesting that a common pathophysiological mechanism may be responsible for the increase in both hormones; in this respect it should be noted that among 14 patients with ACTH-dependent Cushing's syndrome, only in those bearing a pituitary tumour did the concentrations of PRL in the inferior petrosal sinus lateralize to one side of the pituitary and could be stimulated by oCRH (6).

Finally, in 9 patients with ACTH-secreting pituitary microadenomas Crock et al. (5) observed intersinus gradients for basal PRL and GH, in addition to ACTH and $\beta$-endorphin, reflecting the higher hormone level on the ipsilateral side to the tumour.

Concerning the mechanism responsible for the unilateral increase in basal and oCRH-stimulated GH and PRL in blood from one inferior petrosal sinus, at least two possibilities should be considered. First, $\beta$-endorphin, locally synthesized as a cleavage product of POMC in addition to ACTH, might induce the release of GH and PRL by normal pituitary cells adjacent to the ACTH-secreting tumour via a paracrine mechanism, as already suggested in previous studies (5-7). This stimulatory effect, however, should take place at the pituitary level in order to explain the lateralization of the phenomenon; on the other hand, it seems clear from both in vitro and in vivo studies that opioids do not act at the level of the anterior pituitary to induce PRL or GH release (10). Moreover, the absence of an increase in GH and PRL in spite of a clearcut lateralization of ACTH secretion in 4 of our patients points against a $\beta$-endorphin-mediated effect. Alternatively GH and PRL could be co-secreted by the pituitary adenoma, in addition to ACTH. In fact, pituitary tumours that contain one or more hormones, as verified by immunocytoologic techniques, are recognized with increasing frequency; some of them, the so-called silent adenomas may contain several hormones, but do not release the hormonal products in quantities to cause elevated blood hormone levels or clinical symptoms (11).

It cannot be excluded that in these cases blood sampling in the inferior petrosal sinuses would be effective for detecting hormone release.

Whereas the concurrent production of ACTH and PRL by pituitary adenomas has been verified by immunocytoologic and in vitro techniques (12,13), the concurrent production of GH and ACTH has not been so conclusively detected. Two clinical reports show an association of acromegaly and Cushing's syndrome: in one case evidence of ectopic hormone production was obtained (14,15).

In our opinion, the stimulatory effect of oCRH on GH secretion observed in 5 of our patients, might support the possibility of co-secretion of GH by the adenoma; in fact a paradoxical CRH-stimulated GH increase has already been reported in some acromegalic patients (16). The lack of immu-
nostaining for PRL and GH in tumours removed from patients in the present series as well as from patients reported by Crock et al. (5), speaks against tumour production of GH and PRL. However, this hypothesis should not be conclusively rejected, since discrepancies may exist between hormone content and laboratory findings in tumours that are negative by immunostaining.

Although at present the nature of the mechanism responsible for the side-to-side gradients of GH and PRL in blood from the inferior petrosal sinuses in patients with pituitary dependent Cushing's disease remains uncertain, this finding could represent a useful additional signal of the presence and localization of an ACTH-secreting tumour.

References

Received February 9th, 1990.
Accepted April 4th, 1990.

Dr Paola Loli,
Divisione di Endocrinologia,
Ospedale Niguarda Ca Granda,
Piazza Ospedale Maggiore, 3,
I-20162 Milano,
Italy