Vasoactive intestinal polypeptide enhances ACTH levels in some patients with adrenocorticotropin - secreting pituitary adenomas

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Abstract. Vasoactive intestinal polypeptide (VIP) was administered (75 μg iv over 12 min) to 14 patients with Cushing’s disease, 1 patient with Nelson’s syndrome, and 8 normal subjects. VIP induced a significant rise of plasma ACTH levels in 6 patients with Cushing’s disease, from a baseline of 13.2 pmol/l (9.9–18.5 pmol/l) to a peak of 24.5 pmol/l (7.7–18.9 pmol/l), median and range (P < 0.05), and in the patient with Nelson’s syndrome, from a baseline of 260.9 to 461.3 pmol/l. A significant elevation of cortisol levels was also observed, from a baseline of 567 nmol/l (185–842 nmol/l) to a peak of 727 nmol/l (364–1029 nmol/l); P < 0.05. No modifications in plasma ACTH and cortisol levels were noticed in the other 8 patients with Cushing’s disease, or in the normal subjects. In the responsive patients, the median plasma ACTH level reached after VIP was found to be less than that induced by CRH administration. In 2 of the responsive patients, VIP was injected again after successful microadenomectomy and did not then cause changes in ACTH and cortisol concentration. These data demonstrate that VIP specifically stimulates ACTH release in some patients with corticotropinomas but not in normal subjects; the disappearance of such abnormal ACTH responses after successful adenomectomy suggests the presence of specific VIP receptors only on the adenomatous corticotropes.

Vasoactive intestinal polypeptide (VIP), a 28-aminoacid peptide, has been first isolated in the digestive tract and subsequently detected in the central nervous system (Said & Rosenberg 1976), particularly in the hypothalamus (Samson et al. 1979), the hypophyseal portal blood (Said & Porter 1979) and the pituitary gland (Samson et al. 1979). A possible role of VIP as a neuromodulator of the anterior pituitary hormones has been suggested for PRL by in vitro studies in normal rat lactotropes (Ruberg et al. 1978) and in human PRL-secreting pituitary adenomas (Nicosia et al. 1980), as well as by in vivo data in rats (Kato et al. 1978) and men (Ottesen et al. 1981). It has been also demonstrated that the peptide stimulates GH release from human GH-secreting pituitary adenomas in vitro (Matsushita et al. 1981) and in some acromegalic patients (Chihara et al. 1984). Furthermore, preliminary data have indicated that VIP stimulates in vitro ACTH secretion from mouse corticotropin tumour cells (Westendorf et al. 1983; Reisine et al. 1982) and human corticotropinoma cells in culture (White et al. 1982), but not from normal pituitary cells (Rotsztein et al. 1980). In view of these findings, we studied the effect of VIP on plasma ACTH and cortisol levels in patients with ACTH-secreting pituitary tumours and in normal subjects. The magnitude of plasma corticotropin and cortisol increases following VIP administration was compared with that elicited by corticotropin-releasing hormone (CRH).

Patients and Methods

Patients

Fourteen patients with Cushing’s disease (9 women and 5 men, aged 14–51 years), one man with Nelson’s syndrome (aged 32 years) and 8 normal subjects (4 women and 4 men, aged 17–36 years) gave informed
consent to participate to the study. The diagnosis of Cushing’s disease was made on the basis of clinical features, radiological findings (sellar polytomography and high resolution CT scan). Standard hormonal criteria were used, i.e. high urinary 17-hydroxy-corticosteroids (17-OHCS) and free cortisol excretion, exaggerated urinary 17-OHCS response to metyrapone administration (4.5 g orally over 24 h), lack of urinary 17-OHCS suppression after low-dose dexamethasone (2 mg/day for 2 days) but normal suppression after high-dose (8 mg/day for 2 days), absent plasma cortisol circadian rhythm, no plasma ACTH and cortisol response to insulin hypoglycaemia (0.15–0.30 IU/kg iv). Furthermore, in 11 tested patients, a normal or exaggerated ACTH response to ovine corticotropin-releasing hormone (CRH, Bachem Switzerland, 1 µg/kg iv) was also observed.

Mean (± SEM) plasma ACTH and cortisol basal levels were 12.7 ± 1.5 pmol/l (range 3.3–25.9 pmol/l) and 656.9 ± 66.2 nmol/l (range 375–1208 nmol/l), respectively, in the 14 patients with Cushing’s disease and 260.9 pmol/l and 22.1 nmol/l in the patient with Nelson’s syndrome. All patients (except No. 5 and 14) underwent transphenoidal microsurgery: at operation a pituitary microadenoma was identified and resected. Examination by light microscopy with conventional staining techniques and immunohistochemical studies confirmed the presence of an ACTH-secreting tumour in all cases. Seven of the 10 cured patients were re-investigated 2–3 weeks after surgery.

Test protocol
One mg of synthetic VIP (directly given by Prof V. Mutt, Karolinska Institutet, Stockholm, as sterile powder) was dissolved under laminar air flow in 13.3 ml of 0.9% NaCl plus 2% human albumin; 1 ml of this solution (75 µg VIP) was set in sterile, pyrogenic, siliconized vials and promptly frozen at −30°C for not more than 3 months. At the beginning of the test, the content of 1 vial, dissolved in 0.9% saline, was infused over 12 min through an indwelling catheter placed in an antecubital vein. On a separate day, and in random order, a saline infusion was given over 12 min as a control test to patients and normal subjects. After an overnight fast, all subjects were recumbent at bed rest during the test period. Blood samples for cortisol and ACTH determinations were obtained at −30, 0, 5, 10, 15, 20, 30, 45, 60, 90 min through an iv catheter placed in another forearm vein, kept open by a slow saline infusion. In 11 patients with Cushing’s disease plasma ACTH and cortisol response to VIP was compared with the one observed after CRH (1 µg/kg, iv) administration.

Radioimmunoassays
Corticotropin and cortisol levels were determined on unextracted plasma by specific direct RIA methods (Sorin, Italy and Immophase, Corning, USA, respectively). The ACTH assay was specific for ACTH 1–24 and hACTH 1–39; the cross-reactivities of ACTH fragments, γ-and β-LPH, β- and γ-endorphin were below 0.1%. The intra- and inter-assay coefficients of variations were 10% and 15% for ACTH and 4.8% and 6.3% for cortisol; the lower limit of sensitivity of the assays was 3.3 pmol/l and 11 nmol/l, respectively.

For each subject a plasma ACTH response to VIP was defined as present when: a) an increase of at least 50% over the baseline was observed; this value is greater than the 95th centile of ACTH spontaneous fluctuations recorded during saline in patients with Cushing’s disease; b) a net increment greater than twice the maximum intra-assay sp (i.e. 6.6 pmol/l) recorded during the last year.

Data are given as median and range; statistical analysis was performed using the Kruskal-Wallis test.

Results
In normal subjects, VIP infusion did not affect significantly either plasma ACTH or cortisol concentration (Table 1).

In 6 out of 14 patients with Cushing’s disease (Table 1), VIP infusion induced a significant response (P < 0.05) in plasma ACTH from a basal level of 13.2 pmol/l (9.9–18.5 pmol/l) to a peak of 24.5 pmol/l (22.2–37.4 pmol/l). The net increase was 12.5 pmol/l (range 7.7–18.9 pmol/l). The median plasma ACTH peak after VIP was significantly higher (P < 0.05) than that reached during saline (15.6 pmol/l; 5.3–27.7 pmol/l). All these 6 patients showed an increase in plasma ACTH of at least 50% over baseline (mean: 96%; range 55%–140%) and a peak greater than 22 pmol/l.

The pattern of plasma ACTH responses was variable. Plasma ACTH peaked between 10 and 30 min in patients No. 1–4 and at 60 min in patient No. 5. In patients No. 1 and 6 a biphasic ACTH peak occurred.

A significant elevation in median plasma cortisol from a baseline of 567 nmol/l (185–842 nmol/l) to a peak of 727 nmol/l (364–1029 nmol/l) was also observed (P < 0.05). The percent net increase was 52% (range 17%–98%). Plasma cortisol peaks appeared between 15 and 45 min in patients No. 1–4, 6 and at 90 min in patient No. 5. Also, in the patient with Nelson’s syndrome, VIP infusion elicited a marked rise in plasma ACTH from 260.9 to 461.3 pmol/l at 20 min without any significant change in plasma cortisol levels.

In the other 8 patients with Cushing’s disease, no significant modifications in plasma ACTH and cortisol concentration were noticed after VIP.
Comparison of plasma ACTH (pmol/l) and cortisol (nmol/l) levels after VIP and CRH administration in VIP responsive (No. 1–6) and unresponsive (No. 7–14) patients with Cushing's disease and in normal subjects.

<table>
<thead>
<tr>
<th></th>
<th>ACTH Basal</th>
<th>ACTH Peak</th>
<th>Δ</th>
<th>Cortisol Basal</th>
<th>Cortisol Peak</th>
<th>Δ</th>
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<td>VIP responders</td>
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<td>37.4</td>
<td>18.9</td>
<td>557</td>
<td>651</td>
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<td>2</td>
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<td>22.2</td>
<td>7.7</td>
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<td>1029</td>
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<td>25.3</td>
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<td>9.9</td>
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<td>24.0</td>
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<td>727</td>
<td>1838</td>
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<td>0</td>
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<tr>
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<td>552</td>
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P-values: a vs c < 0.05; b vs c NS; d vs e NS; d vs f < 0.01; e vs f < 0.05; g vs h < 0.05; h vs i NS; g vs i < 0.01; l vs m NS; l vs n NS; m vs n NS.

After the injection of CRH (Table 1) to 4 VIP-responsive patients, the rise in median plasma ACTH (28.5 pmol/l) was higher than that induced by VIP (12.5 pmol/l). The magnitude of the median ACTH rise after CRH did not significantly differ between VIP-responsive (28.5 pmol/l) and unresponsive patients (21.6 pmol/l) and was significantly (P < 0.01) greater than that observed in normal subjects (7.9 pmol/l). CRH induced an increase in cortisol levels (595 nmol/l), which was greater than that induced by VIP in responsive patients (183 nmol/l). The magnitude of the median cortisol rise after CRH did not significantly differ among VIP-responsive and unresponsive patients and normal subjects.

No significant differences in basal plasma ACTH and cortisol levels, urinary 17-OHCS and free cortisol excretion were found between VIP responders and non-responders. Moreover, no significant differences were found between VIP responsive and unresponsive patients, as far as urinary 17-OHCS excretion after both low-dose dexamethasone and metyrapone was concerned.

Seven patients with Cushing's disease (No. 2, 3, 7, 8, 10, 11, 13) were re-investigated after successful microadenomectomy. As expected, in all 5 unresponsive patients (No. 7, 8, 10, 11, 13), VIP infusion did not induce any ACTH and cortisol responses. In the other patients (No. 2 and 3), who were ACTH responsive before surgery, plasma ACTH and cortisol levels dropped to low normal values and no responses to VIP occurred, though a plasma ACTH peak of 11.6 and 5.7
pmol/l, respectively, was observed after CRH injection.

In all patients and normal subjects, ACTH and cortisol levels remained unchanged during saline infusion.

Adverse reactions

During VIP infusion, all patients and normal subjects showed transient facial and thoracic flushing, and complained of palpitations and warmth; an increase in pulse rate (up to maximum 125/min) and a decrease in diastolic blood pressure (not greater than 20 mmHg with respect to the initial values) were observed.

Discussion

Although there is increasing evidence that VIP plays a role in the physiological regulation of PRL secretion (Kato et al. 1978; Ruberg et al. 1978; Nicosia et al. 1980; Ottesen et al. 1981), little is known about its possible effect as a neuromodulator of other anterior pituitary hormones secretion in man. In the present report, the influence of VIP on plasma ACTH levels in normal subjects and in patients with ACTH-secreting adenomas has been investigated.

The observation that VIP does not affect ACTH secretion in normal subjects confirms previous results on cortisol levels (Ottesen et al. 1981; Chihara et al. 1984) and is in agreement with the lack of influence of the peptide on ACTH release in vitro from normal corticotropes of both rat (Rotsztejn et al. 1980) and man (Brandi et al. 1984). The most remarkable finding in this report is the demonstration that VIP stimulates ACTH, and cortisol, secretion in some patients with Cushing’s disease and Nelson’s syndrome. It is noteworthy that the magnitude of response to VIP was lower than that elicited by CRH. A smaller ACTH release after VIP than after CRH also has been found in in vitro experiments on pituitary tumour cells from mouse (Reisine et al. 1982) and man (White et al. 1982); in the same studies an additive effect on ACTH secretion by VIP and CRH or by VIP and vasopressin has been reported, suggesting that these peptides act through different stimulatory mechanisms or activation of distinct receptors.

Although we are unaware of similar observations in patients with ACTH-secreting adenomas, previous in vitro studies showed that VIP stimulates ACTH release from human tumourous corticotropes (Oliva et al. 1982; White et al. 1982). It is of interest that the patient with Nelson’s syndrome who markedly responded to VIP in vivo, was the same patient (R.P.), whose corticotropinoma, partially removed by transphenoidal microsurgery two years before, was previously reported to release ACTH in vitro after VIP stimulation (Oliva et al. 1982). The mechanism by which VIP influences ACTH secretion is unknown. However the increase in adenylate cyclase activity in tumourous corticotrope membrane preparations (Oliva et al. 1982) suggests that cAMP plays a role in mediating ACTH release.

In analogy with the abnormal ACTH responses to TRH and GnRH, this rise of ACTH after VIP may be attributable to the presence of altered cell membrane receptors on the adenoma (Ok 1981). The disappearance of such abnormal responses in 2 patients with Cushing’s disease who underwent successful adenomectomy is in favour of this interpretation.

It is worthy of mention that only transformed corticotropes from pituitary tumour of both mouse (Reisine et al. 1982; Westendorf et al. 1983) and man (Oliva et al. 1982; White et al. 1982) are able to release ACTH in response to VIP.

Recently, Jones et al. (1983) reported that VIP induces an ACTH release from neonatal, but not adult, rat pituitary tissue and suggested that tumourous corticotropes which are able to respond to VIP might have regressed to the neonatal state by a process of de-regression. However, it is unclear why this regression process, through abnormal membrane receptors, appears only in some patients with Cushing’s disease.

These data, as well as the abnormal GH stimulation reported in some acromegals (Chihara et al. 1984), indicate that VIP is an agent capable of inducing non-specific hormonal responses in secreting pituitary adenomas. Our preliminary findings suggest that VIP administration, similarly to TRH administration in acromegaly, might have some diagnostic value in patients with ACTH-secreting adenomas. In fact the frequency of ACTH increases after VIP in patients with Cushing’s disease (43%) is greater than those observed after TRH and GnRH tests (Pieters et al. 1982; Ambrosi et al. 1983), and is comparable to the response of GH after TRH occurring in acromegaly (Faglia et al. 1985).
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