

Orthostatic hypotension and vasodilatory peptides in bronchial carcinoma

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Abstract

Aim—To determine whether inappropriately secreted vasodilatory peptides have a role in the pathogenesis of orthostatic (postural) hypotension, a recognised paraneoplastic effect of bronchial malignancies usually attributed to immune mediated destruction of autonomic ganglia.

Methods—Serum concentrations of three vasodilatory peptides, atrial natriuretic peptide (ANP), vasoactive intestinal polypeptide (VIP) and calcitonin gene related peptide (CGRP), were measured in 111 patients with bronchial carcinoma and 35 controls prospectively screened for orthostatic hypotension (>20 mmHg drop in systolic blood pressure on repeated occasions on standing from the supine position) and in whom other causes of this condition were excluded.

Results—Twenty two (20%) patients with carcinoma and two (6%) controls had orthostatic hypotension according to the criteria used. Serum concentrations of ANP, VIP and CGRP were elevated above normal in, respectively, 25 (23%), 10 (9%) and eight (7%) patients with carcinoma and in six (18%), zero and three (9%) controls. There was no correlation between orthostatic hypotension and concentrations of any of the vasodilatory peptides.

Conclusion—Elevated serum concentrations of ANP and CGRP were no more frequent in subjects with bronchial carcinoma than in controls and could not be attributed to the tumour, although there was a possible association for VIP. Orthostatic hypotension was more common in patients with carcinoma, but there was no evidence that the peptides measured played a role in its pathogenesis.

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Orthostatic hypotension is a recognised paraneoplastic effect of bronchial carcinoma,¹² occurring in about 12% of patients with the disease.³ Although immunological injury to autonomic nerves is usually assumed to be responsible,⁴ it has been noted that paraneoplastic orthostatic hypotension is often not accompanied by other features of autonomic dysfunction,³ suggesting that its pathogenesis might not always be neuropathic. One al-

ternative mechanism might involve one or more of a group of vasodilatory peptides occasionally secreted by malignant bronchial tumours.⁴ These include atrial natriuretic peptide (ANP),⁵ vasoactive intestinal polypeptide (VIP)⁶ and calcitonin gene related peptide (CGRP).⁷

The aim of the study was to assess the prevalence of orthostatic hypotension and of abnormal elevation of these three vasodilatory peptides in a series of subjects with bronchial carcinoma and to investigate the relation between them.

Methods

Patients (n = 111) with newly diagnosed, histologically confirmed primary bronchial carcinoma and 35 age and sex-matched controls undergoing bronchoscopic examination, but in whom neoplasia was excluded, were studied. The mean age of the groups was 62 and 63 years, respectively. Of the group with carcinoma, 48 had squamous carcinoma, 16 adenocarcinoma, 16 non-small cell carcinoma which could not be typed further, and 31 had small cell tumours.⁸ Of the 35 control subjects, 10 had chronic bronchitis, nine pneumonia, three had bronchiectasis, and the remainder had no apparent pulmonary pathology.

A full history and examination were performed and biochemical data reviewed on all patients. Subjects were questioned about symptoms of orthostatic hypotension, specifically dizziness or fainting attacks, especially on standing from sitting or lying positions. A careful drug history was taken and any patients taking medication with a hypotensive effect, such as diuretics, anticholinergics, β -adrenoceptor blockers, sedatives, and angiotensin converting enzyme inhibitors, were excluded. Any subjects with clinical or laboratory evidence of disturbances of fluid and electrolytes which might have produced a hypotensive effect were excluded, as were those with diseases of the cardiovascular, endocrine or nervous systems, such as cardiac failure, diabetes mellitus or Parkinson's disease. No patients or control subjects were acutely ill at the time of assessment of blood pressure.

Supine and standing blood pressures were measured using an automated sphygmomanometer with an incorporated printer. Orthostatic hypotension was considered to be present when a drop of 20 mmHg or greater in the systolic blood pressure occurred repeatedly, on separate occasions, on standing from the supine position.⁹ Then, after 15 minutes in a

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Mean (SD) values and 95% confidence intervals (CI) for serum concentrations of the three peptides measured

Subject group	ANP		VIP		CGRP	
	Mean (SD)	95% CI	Mean (SD)	95% CI	Mean (SD)	95% CI
Controls without postural hypotension (n=33)	145.97(168.87)	0-477	24.5 (6.01)	12.5-16.4	115.6(126.0)	0-363
Controls with postural hypotension (n=2)	154.5(118.09)		21.67(2.08)		190.3(172.9)	
Patients with bronchial carcinoma without postural hypotension (n=89)	136.2(213.4)	0-554	28.2(24.0)	0-75.2	106.8(179.9)	0-459
Patients with bronchial carcinoma and postural hypotension (n=22)	125.05(81.92)	0-297	26.82(7.01)	12.2-41.4	91.1(125.9)	0-354

recumbent position, venous blood was drawn with a plastic syringe and immediately transferred to pre-cooled bottles, plastic in the case of CGRP and ANP which tend to adhere to glass, and all containing EDTA and aprotinin to inhibit degradation. After centrifuging at $500 \times g$ for 15 minutes at 4°C , serum was pipetted off and stored at -85°C until assay, which was performed within 12 weeks.

The three peptides were measured using standard radioimmunoassay systems purchased from Incstar Corp., Minnesota, USA (ANP and VIP) and Peninsula Laboratories, California, USA (CGRP).

ANP was extracted using a Sepharose column containing C-terminal specific ANP antibody which was removed from the column with 0.025% HCl and neutralised with 0.1 M NaOH. Sheep anti-ANP serum was added to the samples and standards and incubated overnight at 4°C . Radiolabelled ANP was added to the tubes which were further incubated under the same conditions. Following this, sheep serum pre-precipitated with horse anti-sheep serum and polyethylene glycol were added to the tubes, which were centrifuged. Supernatant was decanted and the radioactivity of the residual pellet measured. The antibody used in the assay showed 100% cross-reactivity with $^{1-28}$ human ANP, but none with adrenocorticotrophic hormone (ACTH) or vasopressin. For assay of VIP, rabbit anti-VIP serum was added to samples and standards and incubated overnight at 4°C . Radiolabelled VIP was added to the tubes which were further incubated under the same conditions. Following this, 0.85% saline and normal rabbit serum pre-precipitated with goat anti-rabbit serum and polyethylene glycol were added to the tubes, which were centrifuged. The supernatant was decanted and the radioactivity of the residual pellet measured.

For assay of CGRP, rabbit anti-human CGRP serum was added to samples and standards and incubated overnight at 4°C . Radiolabelled human CGRP was added to the tubes, which were further incubated under the same conditions. Normal rabbit serum and goat anti-rabbit serum were then added to the tubes, which were centrifuged. The supernatant was decanted and the radioactivity of the residual pellet measured. The antibody used in this assay has less than 0.001% cross-reactivity with calcitonin C-terminal peptide.

Various control procedures were performed to ensure accuracy of the results obtained. Thus, all serum samples were divided into aliquots before freezing so that repeated measurements of peptide concentrations could be

made on the same sample, but using different kits. All assays were performed in duplicate so that any disparity between pairs of results could be quickly recognised and investigated and the assay repeated as necessary. Control serum samples containing known amounts of peptide were repeatedly assayed within and between different kits.

For all assays, radioactivity was measured with a Packard Crystal II gamma counter.

With these assay systems in our laboratory, normal serum concentrations were <150 pg/ml for ANP, <40 pg/ml for VIP and <300 pg/ml for CGRP.

Results

The table shows mean values, standard deviations and 95% confidence intervals for serum concentrations of the three peptides measured.

Of the 111 patients with bronchial carcinoma, 22 (20%) had orthostatic hypotension according to our criteria as did two (6%) of the 35 controls. Serum concentrations of ANP were elevated above normal in 25 (23%) patients with carcinoma and in six (18%) controls. Of these 25 patients with carcinoma, five had orthostatic hypotension, but it was not found in any of the six controls. Serum concentrations of VIP were elevated above normal in 10 (9%) patients with carcinoma, but in no control subjects. Two of the patients with carcinoma had orthostatic hypotension. Abnormally elevated concentrations of CGRP were found with about the same prevalence in both groups—that is, in three (9%) of 35 controls and eight (7%) of 111 patients with carcinoma. None of these three control patients had orthostatic hypotension, but it was present in one of the eight patients with carcinoma.

Although orthostatic hypotension was more common in patients with bronchial carcinoma than in controls, its presence did not correlate with elevated serum concentrations of vasodilatory peptides in either group. Nor, in the 22 patients with orthostatic hypotension and carcinoma, was it associated with any particular tumour type; 11 had squamous carcinoma, seven small cell carcinoma, two adenocarcinoma, and two non-small cell lesions which could not be typed further. Seven of the 10 patients with carcinoma in whom VIP concentrations were abnormally high had squamous carcinoma but, apart from this, there was no correlation between raised peptide concentrations and tumour type.

Discussion

It is generally accepted that orthostatic hypotension may be considered to be present when a subject suffers a fall in systolic blood pressure of at least 20 mmHg on standing from the supine position, consistently, on separate occasions.⁹ It has many causes, but is most often a feature of normal ageing, having been reported to be present in up to 24% of subjects over the age of 65 years,^{9,10} in whom it is usually attributed to physiological deterioration in autonomic function.³ Other causes include autonomic neuropathy, a reduced circulating blood volume or cardiac output, hypovolaemia, acute infection, and a range of drugs.^{9,11}

In addition to the above, orthostatic hypotension also occurs as a paraneoplastic effect of bronchial carcinoma.¹⁻³ Sometimes, it can be attributed to an effect of the disease, such as disturbances of fluids and electrolytes or non-specific severe debility, although it is generally considered to be caused by immune mediated autonomic neuropathy.⁴ This supposition is given some support by the observation of inflammatory and destructive changes in visceral autonomic nerves in paraneoplastic intestinal pseudo-obstruction,^{12,13} although direct evidence for it is lacking and orthostatic hypotension in patients with bronchial carcinoma usually occurs unaccompanied by autonomic dysfunction elsewhere.

An alternative mechanism which has not been assessed as a possible cause of paraneoplastic orthostatic hypotension in patients with bronchial carcinoma involves the inappropriate secretion by the tumour in question of a peptide or peptides with vasodilatory effects. Of the broad range of amine, peptide and protein hormones secreted by bronchial malignancies,⁴ ANP, VIP and CGRP are the most likely to play a role in causing postural hypotension because of their vasodilatory properties. In this study, however, although orthostatic hypotension was certainly more common in patients with bronchial carcinoma than in controls, it could not be attributed to raised serum concentrations of the vasodilatory peptides studied. What, therefore, might it have been caused by? Disturbed metabolism of fluid and electrolytes or effects of vasodilatory drugs are an unlikely explanation, as patients with such confounding features were excluded from the study, as were those with cardiac, renal, nervous, or endocrinological disease likely to interfere with control of blood pressure. It may well be that the mechanism usually suggested, namely autoimmune damage to autonomic nerves, was indeed responsible. The prevalence of orthostatic hypotension in these patients, at 20%, was somewhat greater than in the only comparable study of which we are aware,³ in which it was found in 12% of subjects with the disease, but a postural drop in systolic pressure of 30 mmHg was the criterion for its diagnosis in that investigation, which may well account for the difference. Its prevalence in our control subjects, at 6%, is consistent with previous investigations of its prevalence in subjects of this age.¹⁰

Our figures for the prevalence of raised serum concentrations of the three peptides measured in patients with bronchial carcinoma are difficult to comment on, because previous comparable investigations have been few and sometimes contradictory. For example, although activation of the gene encoding ANP¹⁴ and the peptide itself⁵ have been demonstrated in small cell tumours of the bronchus and raised serum concentrations have been described in occasional patients with small cell carcinomas,^{5,15} the overall prevalence of elevated concentrations in the serum of patients with bronchial carcinoma is unknown. In the present investigation, the fact that there was little difference between the two groups studied in this regard suggests that the elevation might have been unrelated to the presence of a bronchial tumour anyway; there are numerous stimuli to the secretion of ANP, physiological as well as pathological, which might have been responsible and some of the patients in the control group, although free from neoplastic disease, had a variety of inflammatory pulmonary conditions, which might have disturbed its secretion or metabolism. These comments apply equally well to CGRP, despite the fact that it has received more attention in this context than ANP.^{7,16} VIP has also been little studied in the context of bronchial neoplasia, although its inappropriate secretion by such tumours is well documented.⁶ Of the three peptides measured, only this one appeared to show a clear association with the presence of a bronchial neoplasm and it is of interest to note that, as in Said and Faloon's investigation,⁶ most of the tumours in patients with raised VIP concentrations were squamous.

The results of this study support previous observations that orthostatic hypotension is a paraneoplastic effect of bronchial neoplasia, but refute the hypothesis that inappropriately secreted vasodilatory peptides have a role in its pathogenesis.

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