

## Alimentary tract and pancreas

# Bradykinin in carcinoid syndrome

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**SUMMARY** Bradykinin concentrations in peripheral venous blood were measured in seven patients with carcinoid syndrome. The diagnosis was based on typical symptoms and raised urinary excretion of 5-hydroxy-3-indole acetic acid; the carcinoid tumour was verified histologically. Two patients were flushing constantly and the other patients had flushing attacks two to 10 times daily. Several blood samples were taken at weekly intervals from six of seven patients. During 30 sampling procedures the patients were flushing during sampling in 12 instances. Bradykinin was measured by a sensitive solid phase radioimmunoassay technique. Blood bradykinin concentration was normal in all patients. Bradykinin is unlikely to be the vasoactive mediator of flushing.

The symptoms in carcinoid syndrome may be caused by the release of several different biologically active substances into the bloodstream or activation of vasoactive substances in the bloodstream.<sup>1,2</sup> Bradykinin, which is formed in the blood, has traditionally been considered to be one of these substances and perhaps the most important.<sup>3</sup> We have measured bradykinin in peripheral venous blood in a group of patients with carcinoid syndrome using a radioimmunoassay technique and thus attempted to clarify the role of bradykinin in carcinoid syndrome.

### Methods

#### PATIENTS

Seven patients (four women, three men, median age 63 years, range 53-74), were included in the study with symptoms from one to 13 years (median six years). All patients had typical symptoms of carcinoid syndrome with flushing as their main complaint. Two patients were flushing constantly and had diarrhoea, the other patients were flushing two to 10 times daily and had diarrhoea. During the study none of the patients had any bronchoconstriction or

symptoms of cardiac failure. The diagnosis was confirmed histologically and the carcinoid tumour was located in the terminal part of the ileum in all. The patients presented with metastases at laparotomy, six had liver metastases and one mesenteric metastases. The median urinary excretion of 5-hydroxy-3-indole acetic acid was 500 mmol/24 h (range 75-1666 mmol/24 h); normal <50 mmol/24 h.

Blood samples were taken in five patients five times at weekly intervals, in one patient four times at weekly intervals and in one patient only once. The samples were drawn in the morning when the patient was fasting. For a total of 30 sampling procedures, the patients were flushing during sampling in 12 cases. Three separate blood samples were always collected consecutively on each day, bradykinin (240 pg) was added to test tube no 2 before sampling. The collection and processing of samples for blood bradykinin measurements have been described in detail earlier.<sup>4</sup> In summary, blood bradykinin was isolated and measured in the following way: blood from an antecubital vein (free flow samples) was taken within 10 s directly into acetone containing phenanthroline, Polybrene<sup>®</sup> and EDTA. Lipids were removed by extraction with petroleum ether (40-60°C). A final purification was made on QAE-Sephadex A-25 at pH 7.4. The recovery of tracer amounts of <sup>125</sup>I-Tyr<sup>8</sup>-bradykinin was measured in each individual sample. (Overall recovery deter-

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mined for 100 samples of 6 ml whole blood was  $27.8 \pm 6.5\%$  (mean  $\pm 1$  SD).<sup>4</sup> For unlabelled bradykinin, the recovery has been determined as  $82.2 \pm 12.5\%$  (mean  $\pm 1$  SD,  $n=22$ ), when corrected for internal standard recovery.<sup>4</sup> The sensitivity of the assay is 3 pg bradykinin/ml blood, and the between assay coefficient of variation is below 16%. In normal subjects, venous blood concentration of bradykinin is below 5 pg bradykinin/ml whole blood.

## Results

The concentration of bradykinin in whole blood was found to be less than 5 pg/ml in all seven patients, which is normal.

## Discussion

The main symptoms of carcinoid syndrome are flushing, diarrhoea, bronchoconstriction and cardiac failure caused by endocardial fibrosis.<sup>3</sup>

In 1953 Lembeck<sup>5</sup> found that serotonin was released from carcinoid tumours, and it was believed that flushing is mediated by serotonin. It has been difficult to ascribe carcinoid flush entirely to an excessive serotonin release, however, because flushes are not always associated with increased concentrations of circulating serotonin.<sup>2,6</sup>

There is evidence that implicates kinins in the pathogenesis of carcinoid flush.<sup>7-10</sup> In 1964 Oates *et al*<sup>7</sup> found that cutaneous flushes could be produced in five patients with carcinoid syndrome after a single intravenous injection of synthetic bradykinin. The flushes resembled the attacks which occurred spontaneously in these patients. Further, the authors found that nine patients with carcinoid syndrome had a significant increase of the kinin concentration in hepatic venous blood during adrenalin induced flushes. Their experiments also indicated the existence of an enzyme, which can catalyse the formation of a kinin, in metastatic carcinoid tumour tissue. In 1966 Oates *et al*<sup>8</sup> produced evidence for the release of bradykinin in carcinoid syndrome. They postulated that carcinoid tumours release the enzyme kallikrein as a response to stimuli such as adrenalin injection. Kallikrein is normally found in plasma in a precursor form. Since these studies it has been generally accepted that flushing is caused by bradykinin.<sup>11</sup> Bronchoconstriction and perhaps endocardial fibrosis may also be due to either bradykinin or serotonin.<sup>12-15</sup>

The problems encountered in the measurement of bradykinin are numerous and difficult. The human kallikrein-kinin system is closely linked to the clotting, the complement and the fibrinolytic systems, with coagulation factor XII (Hageman's factor) in a

pivotal role. Activation of this factor leads to a kallikrein induced formation of bradykinin. As Hageman's factor is very easily activated and as inactivation of free bradykinin is fast, correct collection and processing of samples require rapid inhibition and removal of the enzymes, which generate and destroy bradykinin. Results of bradykinin measurements, which have been reported, disclose great variations of the concentration, and serious questions have been raised about the results, which have been found in studies, where bioassays and plasma radioimmunoassays have been used.<sup>16-18</sup> Results with the current assays indicate that the normal concentration of blood bradykinin is below 5 pg/ml blood.<sup>1,19</sup>

Although in 30 samples of peripheral venous blood from patients with proven carcinoid syndrome normal blood bradykinin concentration was found to be less than 5 pg/ml whole blood. Twelve of the samples were collected during flushing.

Our methodological data have been reported in detail elsewhere.<sup>1,19</sup> Whole blood is taken directly into acetone within 10 s. The collection tubes are washed with inhibitor solution. These precautions prevent *in vitro* formation of bradykinin. Of course, a whole blood precipitation with an organic solvent leads to a considerable loss of peptide. Therefore, each individual tube contains radiolabelled bradykinin, which serves as a recovery tracer for the individual sample. The recovery is highly reproducible, about 28%. The stability of <sup>125</sup>I-Tyr<sup>8</sup>-bradykinin throughout the procedure has been documented.<sup>1</sup>

Experiments with addition of non-labelled bradykinin before blood sampling have shown an overall recovery of 80%, as corrected by means of recovery tracer data – and recovery of unlabelled bradykinin was controlled in every single blood sampling procedure in the present study. We have also carried out across-procedure stability chromatography studies of endogenous generated bradykinin, which was produced by a delay in addition of acetone, and we found one single compound.<sup>1</sup>

We conclude that patients with carcinoid syndrome do not have raised venous blood concentration of bradykinin. If bradykinin is the mediator of the peripheral vascular reaction known as flushing, one would expect to find raised bradykinin in peripheral blood. It should be taken into account that Oates *et al*<sup>7</sup> carried out their studies 20 years ago. Since then, there has been a tremendous progress in the methodology of bradykinin measurements. They sampled large quantities of blood through a small hepatic vein catheter, and precipitated blood proteins with ethanol, which is less efficient than for example, acetone. The kinin activities in their purified eluates were measured using a bioassay with a very low sensitivity (100 ng/ml blood). With our

present knowledge we must conclude that Oates *et al* overestimated the concentration of circulating bradykinin, mainly because of an *in vitro* activation of the kallikrein-kinin system during sample collection.

It is now known that carcinoid tumours may produce other biologically vasoactive substances such as histamine,<sup>20</sup> prostaglandins,<sup>21</sup> substance P,<sup>22</sup> substance K, and eledoisin.<sup>23</sup> The three latter substances belong to the tachykinins. The relationship between these substances and the clinical manifestations is, however, still not known.

We cannot conclude from this present study that bradykinin is without any importance in the development of the carcinoid syndrome, but our results indicate that bradykinin is unlikely to be the vasoactive mediator of flushing in these patients.

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