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## Note

# Investigation of a peptide fraction in the plasma of pain patients by high-performance liquid chromatography

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In an earlier communication [1], we reported isolation and partial characterisation of a peptide fraction isolated from the plasma of schizophrenic patients. This fraction, when administered intraventricularly into rats, showed profound behavioural changes and analgesia In view of these findings and due to the increasing evidence in the literature that small endogenous peptides like enkephalins, endorphins, etc , are intimately involved with the modulation and perception of pain [2, 3], further studies were carried out to purify similar peptide fractions from the plasma of patients suffering from chronic pain and to determine qualitative and/or quantitative differences with similar fractions from non-pain control subjects. In the course of these studies, we have developed a rapid, sensitive high-performance liquid chromatographic (HPLC) method for the quantitation of the peptide fraction, which could be used as an indicator for the clinical assessment of pain

## EXPERIMENTAL

### *Chromatographic system*

The HPLC analyses were performed on a Waters HPLC system consisting of a

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Model 6000A pump, a U6K injector and a Model 440 UV detector linked to an LKB MultiRac fraction collector, the latter being set to collect fractions in the peak mode The system was operated with a Model 720 system controller linked to a Model 730 data module for the printing of chromatograms and the integration of peak areas (Waters Assoc Milford, MA, U S.A ). A Protein I-60 size exclusion column (30 cm × 7 8 mm I.D ) (Waters Assoc ) was used for the separation

*Patients*

Fifteen patients who had suffered chronic low back pain, in spite of surgical intervention on one or more occasions (including disc removal, phenol injection and laminectomy), were selected for this study The patients were requested to stop taking analgesic drug for 24 h prior to blood sampling A second group of fifteen healthy hospital staff members matched for age (20—40 years) and under no medication formed the control group. Blood samples (5 ml) were collected by venepuncture in heparinised tubes

*Sample preparation*

The procedure for the isolation of the plasma peptide fraction has previously been described [1] Briefly, the method which has been slightly modified involves adding 6% sulphonylsalicylic acid to plasma, centrifuging and decanting the supernatant The solution was diluted with distilled water (5 ml), adjusted to pH 9 and further chromatographed on DEAE Sephadex A-25 The bound material was eluted with 0.1 *M* hydrochloric acid, concentrated by lyophilisation and reconstituted with 0.5 *M* sodium acetate buffer, pH 5.4 (0.4 ml) for analysis by HPLC

*HPLC procedure*

An aliquot (15 $\mu$l) of the reconstituted solution was injected onto the Protein I-60 column. The column was calibrated with standards of known molecular weight prior to use. The column was eluted isocratically at room temperature with 0 5 *M* acetate buffer, pH 5 4 at a flow-rate of 1 ml/min resulting in a back-pressure of about 54 bar (800 p s i.) The absorbance of the effluent was monitored at 254 nm and at 0.1 a.u.f.s.

*Amino acid analysis*

The fraction relating to the major absorbance peak on the chromatogram from a pain patient was collected for amino acid analysis A similar fraction was also obtained from the control group. The material in each case was freeze-dried and the residue hydrolysed with 6 *M* hydrochloric acid (Aristar) for 24 h at 105°C under nitrogen. Amino acid analyses were performed using a Biotronik LC5000 amino acid analyser according to the method of Spackman et al. [4].

## RESULTS AND DISCUSSIONS

A typical HPLC profile of the peptide fraction isolated from the plasma of pain patients and control subjects is shown in Fig. 1. The traces show that apart from several small peaks, there is a major absorbance peak eluting with a

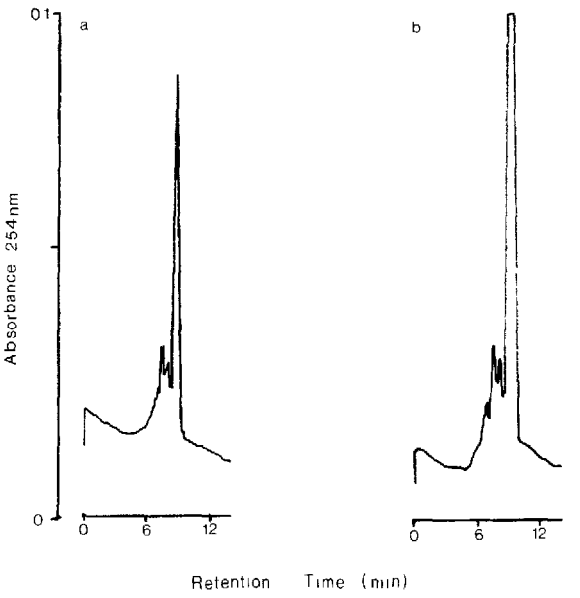


Fig 1 HPLC separation of the peptide fraction isolated from the plasma of (a) a pain patient and (b) a control subject The separation was carried out on Protein I-60 column in 0 5 *M* acetate buffer, pH 5 4 at a flow-rate of 1 ml/min

retention time of about 8 9 min in both the samples from the pain and control groups On the basis of elution volume, the molecular weight of the peptide fraction corresponds to relative molecular mass 1200–1400. However, when the integrated recorded areas of the peak were compared, it was found that there was a significant reduction in the concentration of this peak in the pain sample compared to the control group, when a similar aliquot of peptide material was injected onto the column The area of this peak from pain patients averaged about 53% ($n$ = 15) when compared to the control (100%). The reason for the lower plasma peptide level in pain patients is unclear It may be that either the production of peptide in pain patients is reduced or that it is more rapidly metabolised in response to prolonged pain

Preliminary analysis of the peptide fraction from pain and control samples showed somewhat similar amino acid composition, with some minor differences in the number of amino acid residues For the pain sample, the composition was calculated as $Asp^1$, $Ser^1$, $Glu^4$, $Gly^5$, $Ala^1$, while for the control sample, the composition was $Asp^1$, $Ser^1$, $Glu^3$, $Gly^7$, $Ala^1$ Attempts to match the composition of these peptide fractions with those of other known neuropeptides [5] or fragments of lipotrophin [6] were unsuccessful.

In summary, these results indicate that there is a consistent and significant lowering of a plasma peptide fraction in pain patients with little differences in the amino acid composition, when compared with a similar fraction from control subjects The HPLC method presented here may be useful for the clinical assessment of pain. It remains, however, to be seen whether these peptides are different from other pain-mediating peptides and can act as modulators in chronic pain sufferers

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