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COMBINED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC–RADIOIMMUNOASSAY METHOD FOR THE ANALYSIS OF ENDORPHINS, ENKEPHALINS AND OTHER NEUROTRANSMITTER PEPTIDES

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SUMMARY

Assays for β-endorphin (BE) and its precursors such as β-lipotropin (LPH) in cerebrospinal fluid (CSF), plasma and some tissues have been difficult because of their low concentrations in limited sample volumes, the non-specificity of most antisera. These problems are compounded by the lack of suitable separation methods. Similar problems exist for the enkephalins, tachykinins and dynorphins, among others. This study reports a high-performance liquid chromatographic (HPLC) separation method in which BE and LPH are well separated from each other and which also separates other neuropeptides of interest. The method uses volatile solvents which do not interfere with radioimmunoassay (RIA). Thus by combining HPLC with RIA the method offers, for the first time, a specific assay method for the endorphin, enkephalin and dynorphin families of peptides which does not suffer from the uncertainties in RIA due to cross-reactivities of antisera. Peptide concentrations obtained from the CSF of a small group of chronic pain patients are also presented.

INTRODUCTION

It is desirable to be able to measure the concentrations of specific neurotransmitter peptides in plasma and in cerebrospinal fluid (CSF) in order to understand more fully the role that these peptides play in the transmission and modulation of, among other sensations, pain. One of the major problems in the quantitative analysis of putative neurotransmitters (such as the enkephalins and the endorphins) is that of specificity. It is necessary to use radioimmunoassay

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(RIA) techniques to achieve the necessary sensitivity, and most antibodies available are not totally specific. We have recently reported the production of antisera specific for the enkephalin pentapeptides [1] and a combined high-performance liquid chromatographic–radioimmunoassay (HPLC–RIA) procedure for analysis of these two pentapeptides [2]. In the latter, specificity for the pentapeptides was obtained by use of the above antiserum, and [Leu^5]enkephalin (LE) was separated from [Met^5]enkephalin (ME) using an HPLC separation method.

A similar difficulty exists for the pro-opiomelanocortin (POMC) family. Most antisera rasied to β-endorphin (BE) cross-react 100% with β-lipotropin (LPH) and to a lesser extent with other fragments of POMC [3,4]. Some specific antisera have been reported [5] with less than 1.5% cross-reactivity with LPH. Even in such a case, the results of assays undertaken with these antisera must be interpreted with care, since LPH and POMC are precursors of BE and may exist in much greater concentration than BE itself. In that case, the actual concentrations of POMC, LPH and BE will be unknown and BE-like immunoreactivity only will be measured, the sum of all the cross-reacting peptides. Gel filtration has been used to separate these peptides [3,6] but the low flow-rates mean long separation times of up to 24 h, and the separation is frequently poor. Both N- and C-terminal specific antibodies have been used in double assays [7]. This adds to the time and cost of the assay and may still leave uncertainties when more than two cross-reacting peptides are present.

Separations by HPLC of some of the members of the POMC family of peptides have been published before [8–11]. Some of these are based on the triethylamine phosphate (TEAP) system [8] pioneered by Rivier [12], while others rely on fluorinated alkyl acids as ion-pairing reagents [9,10,13]. TEAP or other low-pH non-volatile systems may interfere with many RIA systems by disturbing the pH or introducing other salts. These and other reported separations of BE and LPH do not provide clear baseline separation of these peptides to allow their use in routine RIA of CSF or plasma samples, nor do they offer separations of other peptides such as the enkephalins, dynorphins, somatostatins and tachykinins. An ion-exchange HPLC method has been published [14,15] which achieves a separation between BE and LPH; the method was not used to separate other peptides such as those mentioned above.

The aim of this work was to develop a separation system that would separate many peptides with high recovery of biological activity, in particular the LE/ME and the BE/LPH pairs such that a sensitive RIA could then be used quantitatively.

EXPERIMENTAL

Apparatus

A Varian 5500 ternary HPLC solvent delivery system (equipped with a Rheodyne 7125 manual injector with a 470-μl loop) and a Varian 2050 variable wavelength UV monitor (10 mm pathlength, 8 μl volume) were used (Varian Assoc., Warrington, U.K.). The columns used were a Merck (BDH, Poole, U.K.) LiChrospher 300 RP-8 10-μm (250×4 mm) in a manual cartridge holder with a 4×4 mm guard column of the same material, or a Waters (Millipore-Waters,

TABLE I

HPLC SYSTEMS USED

System	Column	Guard column	Solvent A	Solvent B	Solvent C	Gradient
TEAP/μBondapak	Waters μBondapak C_{18}, 10 μm, 300×4 mm	Whatman Pellicular C_{18}, 70×2 mm	0.1 *M* TEAP pH 3.0 in water	0.1 *M* TEAP pH 3.0 in acetonitrile	None	25–40% B, linear, 15 min
TFA/μBondapak	Waters μBondapak C_{18}, 10 μm, 300×4 mm	Whatman Pellicular C_{18}, 70×2 mm	0.225% TFA in water	0.225% TFA acetonitrile	None	35–40% B, linear, 7.5 min
TFA/LiChrospher	Merck LiChrospher 300A C_8, 10 μm, 250×4 mm	Merck LiChrospher 300A C_8, 10 μm, 4×4 mm	Water	Acetonitrile	1% TFA in water	20–48% B at 5% C, linear, 20 min, then 5–20% C at 48% B, linear, 5 min

Harrow, U.K.) μ-Bondapak C_{18} (300×4 mm) with a guard column (70×2 mm) containing a pellicular RP C_{18} media (Whatman LabSales, Maidstone, U.K.). The flow-rate used throughout was 1 ml/min. Fractions were collected using a Gilson 201 fraction collector and were dried using an Edwards (Crawley, U.K.) Modulyo freeze drier equipped with a spin-freeze accessory. Stacking home-made rotors (perspex) capable of holding 90 2-ml tubes each were used in place of the supplied aluminium rotor. RIA assays were separated (see below) using a Beckman J6B refrigerated centrifuge with a JR3.2 rotor.

Reagents

HPLC solvents were water, HPLC-grade S acetonitrile (Rathburn, Walkerburn, U.K.), trifluoroacetic acid (TFA) (Aldrich, Gillingham, U.K.), passed through a Sep-Pak C_{18} cartridge (Millipore-Waters) made 1% in water, and 0.1 *M* orthophosphoric acid adjusted to pH 3.0 with triethylamine (TEAP). All solvents were filtered and vacuum-degassed using a 0.45-μm Nylon filter (Anachem, Luton, U.K.). Peptides were obtained as amino acid-analysed equivalents from Peninsula Labs. Europe (St. Helens, U.K.) for porcine and human LPH, and Cambridge Research Biochemicals (Harston, U.K.).

HPLC conditions

Gradient elution was carried out at room temperature as detailed in Table I and in the figure legends. Fractions were collected for RIA in 2-ml screw-capped polypropylene tubes (Sarstedt, Beaumont Leys, U.K.) every 30 s, and dried under vacuum in the spin-freeze to prevent bumping.

Extraction

CSF from patients undergoing surgical pain relief was collected into cold polystyrene tubes containing 20 μl formic acid per ml CSF, and extracted immediately or frozen at $-20°C$. Extraction was by Sep-Pak C_{18} cartridges (Waters) using a pump flow-rate of 1 ml/min. The cartridges were pre-wetted with 4 ml of methanol–water–TFA (80:20:0.1, v/v/v) and 3 ml of 0.1% TFA, followed by application of a 1- or 2-ml sample. The cartridges were then washed with 4 ml of water, 2 ml of 0.1% TFA, and eluted with 1.6 ml of methanol–water–TFA (80:20:0.1) into polypropylene tubes [2]. These samples were then vacuum-dried, redissolved in HPLC starting solvent (400 μl) and analysed by HPLC.

Radioimmunoassay

Enkephalin assays were carried out as described previously [2]. The assay buffer was 0.02 *M* sodium phosphate, pH 7.4, containing 0.14 *M* sodium chloride, 0.6 m*M* thimerosal and 1.4% (v/v) bovumin [22% bovine serum albumin (BSA) sterile solution; Sigma, Poole, U.K.]. Standards or unknowns (the appropriate dried HPLC fractions redissolved in assay buffer) were prepared in polystyrene tubes with 100 μl [^{125}I]LE (1.5 fmol; 1200 cpm) and 100 μl of the appropriate antiserum dilution. The ^{125}I is incorporated in the tyrosine phenyl ring of the enkephalin – the same as used for the coupling to BSA for antiserum preparation. This does not affect the RIA adversely [1,2]. Buffer was added to each tube to a final incubation volume of 1 ml. Incubation was for 48 h (antiserum No. 629 [1,2]) at 4°C, then separation of bound from free was achieved by addition to each tube of 100 μl of a slurry of Norit OL charcoal (0.1 g/ml), dextran grade C (0.01 g/ml) in assay buffer. The tubes were immediately centrifuged for 10 min at 1400 *g* and 4°C. Bound and free fractions were separated and counted.

Endorphin assays (BE and LPH) were carried out using an anti-BE antiserum purchased from Amersham International (Amersham, U.K.) which had 100% cross-reactivity with LPH. An anti-BE antiserum developed in our laboratories was also used which had 1% cross-reactivity with LPH. The appropriate dried HPLC fractions were redissolved in buffer [0.05 *M* sodium phosphate, pH 7.4, containing 10 m*M* EDTA and 0.2 mg/ml BSA (RIA grade 5, Sigma); RIAB]; [Tyr^{27}]-^{125}I-labelled BE (Amersham International) was added (3000–5000 cpm per tube), followed by antibody solution (50 μl of Amershams recommended stock solution) in a total volume of 500 μl. After thorough mixing, the tubes were incubated at 25°C for 1.5 h and separation effected using a modification of the centrifugal gel permeation method [16]. Columns of well washed Ultrogel AcA54 (LKB, South Croydon, U.K.) were made up in 1-ml syringe barrels with a hydrophilic porous polyethylene frit (Radley, Sawbridgeworth, U.K., treated with concentrated sulphuric acid and thoroughly washed until neutral). These columns were then pre-spun at maximum acceleration for 10 s until a speed of 1000 rpm (200 *g*) was reached in our Beckman J6B centrifuge with JR3.2 head. After the pre-spin the gel appeared whitish but not cracked. Aliquots of 150 μl were then taken from the incubation tubes and applied to individual columns in triplicate, which were then spun at 1000 rpm (200 *g*) for 2 min. The column eluates were collected in the polystyrene tubes (100 × 13 mm) that the columns were held

in; the tubes were then counted in a Kontron Gammamatic B gamma counter. In this assay method, free tracer remains in the gel in the column, while antibody-bound tracer passes through the column and is collected.

Somatostatin (SS) was assayed using an anti-SS antiserum and iodinated tracer purchased from Amersham International. The protocol provided by Amersham was followed exactly. This antiserum is reportedly specific for somatostatin-14 (SS14) and somatostatin-28 (SS28), although on assay of HPLC eluates, we have found evidence of not only SS14 and SS28, but also another, as yet unidentified, cross-reacting peptide.

RESULTS

Reversed-phase HPLC was chosen as being the most likely method to provide a separation between BE and LPH. Previously published binary gradient methods using TEAP [8] or fluorinated alkyl acids such as TFA and heptafluorobutyric acid [9,10,13] as ion-pairing reagents were tried and found to be unsuitable either because the non-volatile buffer systems interfered with our RIA or the resolution was insufficient or both. Preliminary experiments with the wide-pore LiChrospher 300A C_8 10-μm column showed that at low TFA concentrations (0.05%) BE could be eluted on an acetonitrile gradient whereas LPH was retarded completely. Higher concentrations of TFA (up to 0.25%) allowed elution of the LPH. This behaviour could not be reproduced with μBondapak C_{18} (Waters) or Micropak C_{18} (Varian) columns. At 0.05% TFA, the smaller peptides from the enkephalin and dynorphin families, as well as substance P (SP), SS and others, could be separated on a steep acetonitrile gradient of 20–48% over 20 min. Thus a two-step gradient of 20–48% (linear) acetonitrile at 0.05% TFA over 20 min, followed immediately by a linear increase of TFA to 0.20% over 5 min at 48% acetonitrile produced the desired separation of BE from LPH, together with the separation of the other peptides listed.

Fig. 1 shows the separation achieved using the LiChrospher 300 RP-8 column for a mixture of six commercially available peptides thought to be involved in pain transmission/modulation. This UV trace at 280 nm shows that the peaks are sharp, with a minimum of tailing, even for the higher-molecular-mass compounds that elute late such as BE and LPH, whose molecular masses are 3465 and 10 000, respectively. This wavelength of 280 nm was chosen rather than 210 nm because of the absorbance at the lower wavelength of TFA, which would give rise to an unacceptable baseline drift. Table II shows the elution times using this system obtained for these and other neuropeptides. The separations are complete with the exception of the pair ME/dynorphin 1-6, where there is a slight overlap. This could be improved by using a lower initial acetonitrile concentration and a shallower gradient. Fig. 2 shows the RIA profile of a mixture of BE and LPH separated with this method.

It was found that as the column aged, the retention times were decreased. This could be corrected by decreasing the starting concentration of acetonitrile to restore the separation to its original form. Regular checks of the retention times using standards were carried out. After retention time checks with UV–visible

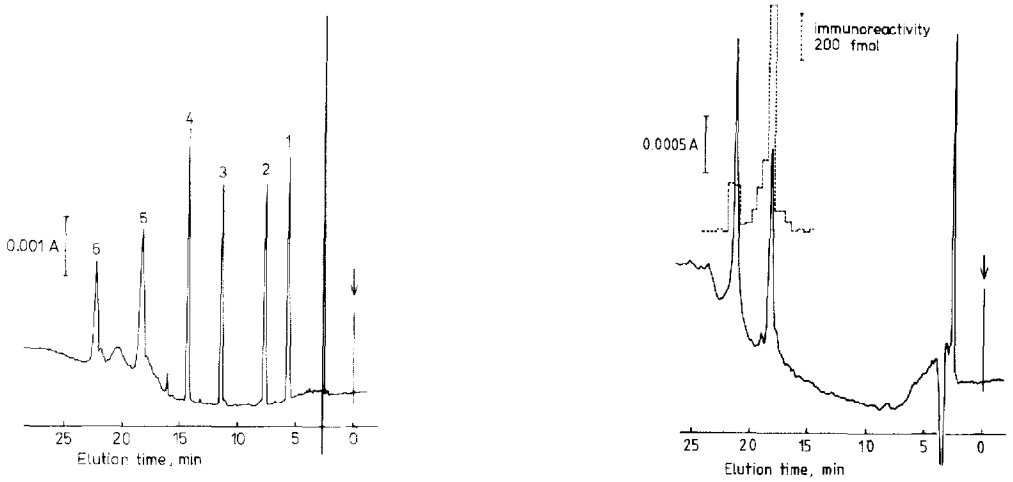

Fig. 1. HPLC of a mixture of synthetic peptides on a Merck LiChrospher 300 RP-8 10-μm cartridge. The gradient used was as described in Table II, but with a 15-min acetonitrile gradient. Flow-rate, 1.0 ml/min; UV absorbance monitored at 280 nm at a sensitivity of 0.01 absorbance units full scale deflection. Peaks: 1 = ME (0.2 μg); 2 = LE (0.2 μg); 3 = cholecystokinin (0.2 μg); 4 = SS (0.2 μg); 5 = BE (2.5 μg); 6 = LPH (1 μg); other peaks are injection artefacts and impurities.

Fig. 2. Superimposed chromatogram and RIA profile of a mixture of BE and LPH. Injection was 400 μl containing BE (1 μg, 289 pmol) and porcine LPH (0.8 μg, 80 pmol). Fractions (500 μl) were collected, 10 μl of each fraction dried and redissolved in RIAB (1.0 ml). Duplicate 300-μl aliquots were assayed for BE immunoreactivity using the Amersham antibody. Arrow marks point of injection. Conditions as for Fig. 1. Recovery of BE, 100% and of LPH, 87%.

quantities (100–400 pmol) of peptides, the column and injector loops were washed thoroughly by running two blank gradients. There was no detectable carry-over of peptides under these conditions, as determined by RIA of BE and enkephalins. If a blank run and loop washing was not carried out, however, spuriously high RIA values were obtained. The column currently in daily use is eight months old and is reversed according to the suppliers recommendations every two to four weeks.

Effect of buffers on RIA

Table III shows that TEAP interfered severely with the BE RIA used in this study, even when the buffer molarity was increased to overcome the pH or ionic effects of the TEAP. The assay sensitivity was severely reduced, and the total binding reduced by 50%. The volatile TFA–acetonitrile systems did not interfere with any of the RIAs used in this study (BE/LPH, SS, enkephalins).

Recovery of peptides

The mean recoveries obtained for BE and LPH from the Sep-Pak procedure and the various HPLC systems are detailed in Table IV. These show that an almost quantitative recovery of material was obtained from the Sep-Pak procedures and from HPLC methods using TFA ion-pairing. From 1000 fmol of BE or

TABLE II

RETENTION TIMES OF VARIOUS NEUROTRANSMITTER PEPTIDES ON LICHROSPHER 300 RP-8 10-μm HPLC COLUMN

LiChrospher 300 RP-8 10-μm column (250×4 mm) with guard column (4×4 mm) of the same material. Flow-rate, 1 ml/min, 470-μl loop. Gradient, 20–48% acetonitrile, linear over 20 min at 0.05% TFA, then 0.05–0.20% TFA at 48% acetonitrile over 5 min.

Peptide	Retention time (min)
Lys-Lys-Gly-Glu (MPF)	2.92
[Met^5]Enkephalin	6.40
Dynorphin 1-6	6.90
[Leu^5]Enkephalin	8.40
Dynorphin 1-8	10.35
Neurokinin A	11.50
Cholecystokinin-8	12.85
Substance P	14.00
Somatostatin-14	15.65
Neurokinin B	16.80
Somatostatin-28	17.05
β-Endorphin	20.70
β-Lipotropin	25.00

TABLE III

EFFECT OF TEAP ON ENDORPHIN RIA

A blank HPLC gradient was run, and fractions were collected from the centre of the run. The column was μBondapak C_{18}, solvent A was 0.1 *M* TEAP pH 3.0, solvent B was 0.1 *M* TEAP pH 3.0 in acetonitrile. The gradient was 25–40% acetonitrile linear over 15 min. The fractions were dried under vacuum and redissolved in either 50 or 100 m*M* RIAB. RIA was carried out (see Experimental) with none or 100 fmol BE added to the assay tubes.

Buffer concentration (m*M*)	Control		+TEAP	
	B_0*	100 fmol**	B_0*	100 fmol**
50	57.2	0.246	26.9	0.179
100	47.3	0.211	30.0	0.067

*B_0 is expressed as a percentage of the total counts applied to each assay column.
**This figure represents the fraction of B_0 binding displaced by 100 fmol BE; i.e. a measure of assay sensitivity.

LPH, 90–100% was recovered from the LiChrospher HPLC system. The system using TEAP showed a much lower recovery of 50% on both RIA (allowing for interference) and from labelled BE.

In order to further check the recoveries of peptides on the HPLC–RIA system, mixtures of LE, ME and BE were injected in total volumes of 400 μl, fractions collected, dried and assayed. Amounts injected were 10–500 fmol (two to four injections for each quantity), and the results were subjected to linear regression analysis. Table V summarises the regression analysis. Recovery of ME was lower

TABLE IV

RECOVERY OF BE AND LPH FROM SEP-PAK AND HPLC

Pooled CSF was spiked with [^{125}I]BE or with 1000 fmol BE or with 1000 fmol LPH. The CSF was extracted as detailed in the text with Sep-Pak C_{18} cartridges, the eluate dried and counted, assayed using RIA or subjected to HPLC and RIA. HPLC systems and gradients as described in Table I.

System	Recovery (%)	
	[^{125}]BE	RIA (BE, LPH)
Sep-Pak	95	95–105
TEAP/μBondapak	50	45– 55
TFA/μBondapak	95	80–100
TFA/LiChrospher	95	90–100

TABLE V

LINEAR REGRESSION ANALYSIS OF PEPTIDE RECOVERIES

Two to four injections of mixtures of LE, ME and BE (10–500 fmol each) were made onto the ternary LiChrospher HPLC system as described in Table II. Fractions were collected and dried, and the appropriate assays carried out. Linear regression analysis of recovery as a function of load was carried out.

Compound	Recovery (%)	r
ME	78	0.964
LE	104	0.985
BE	91	0.999

and more variable than that for LE or BE; this was because precautions to prevent oxidation of the methionine residue were not taken. Our anti-enkephalin antiserum is directed towards the whole pentapeptide sequence [1] and is extremely sensitive to oxidation of ME, failing to cross-react with the oxidised form [1]. There is no evidence that oxidation of the Met^5 residue of BE affects its RIA, if it occurs. Recovery of BE was 91%, and 104% for LE.

CSF concentrations

The TFA/LiChrospher HPLC–RIA system was used to investigate the concentrations of a number of peptides thought to be involved in pain transmission and/or modulation in the CSF of patients with chronic pain. These patients were all undergoing radiofrequency or alcohol lesions of the pituitary. CSF samples were obtained (from between vertebrae C1 and C2) prior to the procedure, then 10 min after electrical stimulation of the pituitary, and again 10 min after the lesion had been made. Table VI shows the results obtained for the enkephalins, SS, BE and LPH from seven of these patients and one patient suffering from congenital inability to feel pain.

The TFA/LiChrospher gradient system resolves many more CSF peptides than the TEAP or TFA/μBondapak systems. For example, Fig. 3 shows a typical chromatogram obtained with the TFA/μBondapak system from Sep-Pak-extracted

TABLE VI

CONCENTRATIONS OF PEPTIDES IN CSF FROM PAIN PATIENTS

Samples of human CSF (1–2 ml) were extracted as described in Experimental, dried and subjected to HPLC using the LiChrospher RP-8 column and ternary gradient system as in the legend to Table II. Fractions (0.5 ml) were collected, dried in vacuo, redissolved in the appropriate buffer and assayed for the various peptides as described in Experimental.

Patient		Concentration (fmol/ml of CSF)				
		LE	ME	BE	LPH	SS
AOV	Pre	14	14	35	31	<1
	Stimulation	12	18	48	12	<1
	Lesion	6	78	58	10	10
BOH	Pre	<3	<5	63	14	<1
	Stimulation	<3	<5	38	27	2
	Lesion	6	<5	1360	63	5
CQV	Pre	4	23	<4	<4	<1
	Stimulation	4	42	43	180	<1
	Lesion	3	31	<4	53	<1
DWR	Pre	<3	<5	385	30	4
	Stimulation	<3	94	126	43	2
	Lesion	6	108	381	119	3
FBJ	Pre	10	9	9	<4	1.5
	Post alcohol	14	6	<4	<4	1.2
GHJ	Pre	25	76	49	35	<1
	Post alcohol	4	51	96	63	12
HHR	Pre	13	47	33	<4	4
	Post alcohol	20	22	26	<4	31
EHJ	Congenital analgesia	<3	<5	210	20	76

CSF. This patient was undergoing treatment for chronic pain which involved radiofrequency lesions made in the pituitary. As can be seen, a large unresolved peak appears at the solvent front, with few further peaks. The elution positions of BE and LPH are marked with arrows. In contrast, Fig. 4 shows a trace of the same sample of CSF using the TFA/LiChrospher gradient system in which a large number of well resolved peaks can be seen. Again, BE and LPH elution positions are marked. Of great interest is the observation that some of these peaks are only present in CSF after electrical stimulation of the pituitary.

DISCUSSION

The method of Seidah et al. [8] was initially used in the separation and analysis of the endorphin peptides using TEAP and a Waters μBondapak RP C_{18} column. The system was optimised for the separation of BE and LPH, and the maximum separation that could be obtained between BE and LPH was 1.5 min using a shallow acetonitrile gradient. This system, however, gave low recoveries

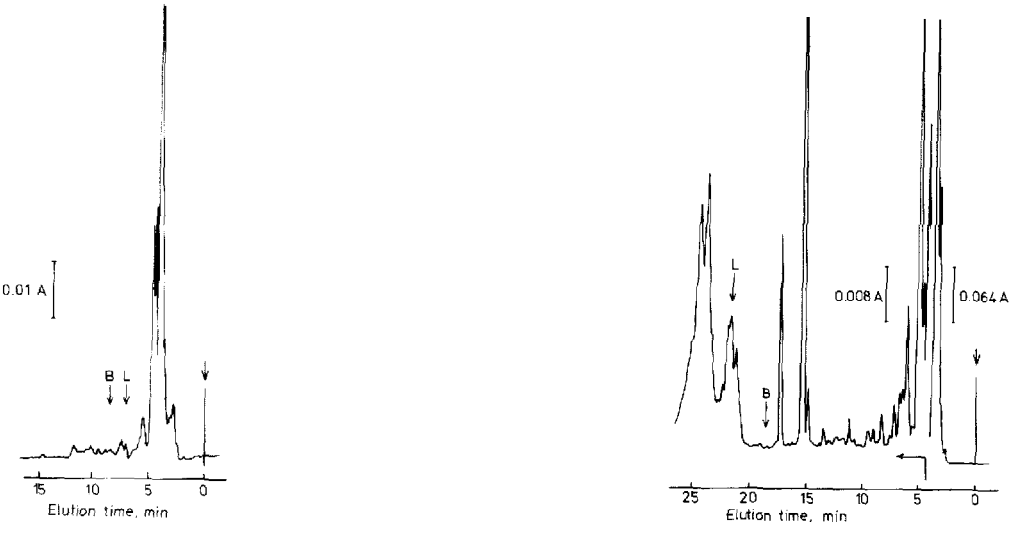

Fig. 3. HPLC of Sep-Pak-extracted CSF on μBondapak C_{18}. CSF (2 ml) was Sep-Pak-extracted, redissolved in starting solvent (500 μl) and injected onto the column [μBondapak C_{18} (300×4 mm) with C_{18} pellicular guard column (70×2 mm)]. Solvent A, 0.225% TFA; solvent B, 0.225% TFA in acetonitrile. Starting composition, 35% B; gradient, linear to 40% B over 7.5 min. Flow-rate, 1 ml/min. Detector, UV at 280 nm. B and L are elution positions of BE and LPH.

Fig. 4. HPLC of Sep-Pak-extracted CSF on the TFA/LiChrospher system. Sample as for Fig. 3, conditions as for Fig. 1. B and L are elution positions of BE and LPH. Note change of scale expansion at 4 min.

(Table IV), broad peaks and poor resolution of the smaller peptides. There were large losses of endorphin which may have been due to non-specific adsorption on the column. These losses, combined with the non-specific effect of TEAP on the RIA for BE and LPH (Table III), made this system unsuitable for use in combined HPLC–RIA analysis of these and other peptides.

The second system evaluated was an acetonitrile–TFA system using the same μBondapak RP C_{18} column. After optimisation of the TFA concentration, a separation of 2.0 min between BE and LPH was obtained at 0.225% TFA and a 35–40% acetonitrile gradient over 7.5 min. Starting the gradient at a lower acetonitrile gradient was found to give a better resolution for low-molecular-mass peptides such as the enkephalins, but at the expense of a loss of separation of the BE/LPH pair. In general, these TFA/μBondapak systems gave good recoveries, but the separation was not adequate given the peak tailing that occurred.

The LiChrospher 300A RP-8 column combined with the ternary gradient system described gave the best overall recovery and separation and was used throughout for the analysis of neuropeptides from human CSF.

A major advantage of this combined HPLC–RIA assay method is that many peptides can be assayed from one CSF sample of 1 or 2 ml. This is due to the separation of the peptides during HPLC and greatly reduces the amount of CSF required to carry out assays on a large range of neuropeptides. Furthermore, the method resolves many UV–visible peptides, the concentrations of some of which

are very obviously affected during the neurosurgical procedures undergone by these patients. Some of these fractions are currently under investigation.

It is also of interest that the range of peptide concentrations reported here is so large. We have found that two patients had undetectable levels of ir-BE and ir-LPH (ir=immunoreactive) after ablation of the pituitary with alcohol or radiofrequency lesion, whereas three others showed increases (Table VI). The radiofrequency lesioning procedure appears to cause an increase in ir-LPH, ir-BE and ir-ME in CSF, may affect ir-SS, but appears to have no effect on ir-LE concentrations. One patient showed a very large increase of ir-BE, together with a rise in ir-LPH and no change in the smaller peptides. These increases in ir-BE, presumably released as a result of the stimulation/lesion procedure, may account for the decrease in pain obtained by these patients. All the patients reported here obtained some pain relief. Pituitary alcohol ablation, on the other hand, seems to cause a decrease in ir-ME and rise in ir-SS. The other peptides measured showed no trend. These results will be reported on in detail when sufficient numbers of samples have been analysed. The mechanism of release is unclear, and may arise from an increase in processing of precursors (perhaps accounting for the occasional decreases in LPH concentration observed) or from a stimulation of peptide release from the pituitary.

This ternary HPLC method, combined with readily available RIAs, provides a specific, sensitive assay for many neuropeptides. There may, of course, be other unknown cross-reacting peptides eluting at exactly the same time as the peptides being assayed, but this is unlikely, and the combination of HPLC with RIA provides much greater specificity than otherwise available. In particular BE and LPH can be assayed without interference with each other, and the enkephalins, ME and LE, can also be separately assayed without mutual interference. Only a small sample is necessary to assay as many peptides as desired.

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