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Measurement of plasma serotonin by high-performance liquid chromatography with electrochemical detection as an index of the *in vivo* activity of fluvoxamine

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method is described for the measurement of plasma serotonin concentrations. Sample preparation is by a simple solid-phase extraction using C_{18} columns. An isocratic separation is used with electrochemical detection. The application of the method to the measurement of plasma serotonin concentrations following the administration of fluvoxamine (a serotonin re-uptake inhibitor), maprotiline (a noradrenaline re-uptake inhibitor) and placebo to normal subjects for a seven-day period is reported. Fluvoxamine significantly decreases plasma serotonin over this time period in a linear fashion. No effect on plasma serotonin was seen for maprotiline or placebo. Plasma serotonin concentrations can be used to monitor compliance with fluvoxamine therapy.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter located primarily in the enterochromaffin cells of the intestine, in the brain and in blood platelets. It is believed to play an important role in the pathophysiology of mood disorders, and a number of antidepressant drugs have been developed which act primarily on the serotoninergic pathways, *e.g.* clomipramine. Fluvoxamine is a specific serotonin re-uptake inhibitor [1] originally designed as an antidepressant but which has anxiolytic properties in panic disorders [2] and obsessive compulsive disorders [3]. Fluvoxamine has also been observed to produce anxiety in normal volunteers [4], being most pronounced after four days of treatment. As part of a study to investigate whether specific monoamine re-uptake blockers can induce anxiety the psychopharmacological effects of fluvoxamine were compared with those of maprotiline, a potent noradrenaline re-uptake inhibitor, and placebo in normal subjects. Determination of serotonin concentrations in whole blood [5,6] have been used as a measure of cerebral sero-

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toninergic activity. Isolation of platelets has the potential to cause disruption of the platelets and release of their contents. Whole blood is therefore used by some workers [5–8] as an indirect measure of platelet serotonin, although platelet heterogeneity may have some effect on the overall results. The concentration of serotonin in platelet-free plasma accounts for only 0.1% of that found in whole blood, but plasma is more easily obtained and stored. In this study serotonin concentrations in platelet-rich plasma were measured to determine whether this is an equally sensitive indicator of central serotonin function.

A number of methods for the measurement of serotonin in blood using a variety of techniques have been described in the literature including fluorimetric methods [9], gas chromatography–mass spectrometry [10] and radioimmunoassay [11]. The most commonly used technique, however, is high-performance liquid chromatography (HPLC) with either fluorescence [12–14] or electrochemical [15,16] detection. In these assays sample preparation consisted of protein precipitation with either perchloric acid [8,13,14] or trichloroacetic acid [15] which does not have the benefit of an extraction step in removing potential interfering substances. Korpi [16], using a HPLC method with fluorescence detection, recommended the use of carbon dioxide pretreatment to inhibit any degradation of serotonin by oxyhaemoglobin. Babcock *et al.* [8], in a comparison of methods, found that perchloric acid did not affect serotonin in whole blood, as an excellent correlation was obtained between the method of Korpi [16] and that of Anderson *et al.* [14]. Liquid–liquid extractions [7] can be time-consuming and labour-intensive unless a derivatising autosampler is used. Solid-phase extraction is relatively simple and inexpensive to use and can be applied to a large number of compounds of interest in the biomedical sciences. In this paper we describe a simple, specific method for the determination of plasma serotonin using reversed-phase HPLC with electrochemical detection following solid-phase extraction. The application of plasma serotonin measurements to assess the *in vivo* effects of the serotonin re-uptake inhibitor fluvoxamine is described.

EXPERIMENTAL

Subjects

Eight normal, healthy volunteers, aged between 20 and 38 years, four males (mean age 27.5 years) and four females (mean age 22.75 years) took part in the study. Subjects were given three seven-day oral treatments with 100 mg of fluvoxamine, 75 mg of maprotiline and placebo all formulated as identical capsules and each given at 11.00 a.m. The study was carried out double blind with treatment periods allocated in random sequence as shown in Table I. There was a period of at least one week between treatments to avoid carryover effects. Blood (2 ml) was taken for measurement of plasma serotonin, in the morning before medication, on days 1, 4 and one day after the last dose (day 8) for each treatment period.

Samples

Blood (2 ml) was collected into lithium heparin tubes containing 200 μl of pargyline (250 μmol/l) to inhibit the action of monoamine oxidase on serotonin. Samples were centrifuged within 2 h and the plasma stored at −20°C until assayed.

Equipment

An ACS quaternary gradient pump was used in isocratic mode with helium degassing of all solvents. Injection was via a Rheodyne valve fitted with a 20-μl loop (Jones Chromatography, Hengoed, UK). A C_{18} ODS Hypersil column was used (APEX cartridge column, 150 mm × 4.6 mm I.D., Jones Chromatography). No guard column was used but an in-line filter was fitted between the valve and the column. The electrochemical detector was an LCA 16 (EDT Instruments, London, UK) and data were collected in a CHROMJET integrator (Spectra-Physics, Hemel Hempstead, UK).

Reagents

The extraction buffer was a carbonate–bicarbonate buffer (2.65 g of sodium carbonate and 18.9 g of sodium bicarbonate in 500 ml of deionised water adjusted to pH 9.2). The internal standard chosen was 5-hydroxyindole (75 μmol/

TABLE I

SUBJECT DETAILS AND TREATMENT PATTERN

Subject No.	Sex	Age	Week 1	Week 2	Week 3
1	M	26	Maprotiline	Fluvoxamine	Placebo
2	M	20	Placebo	Fluvoxamine	Maprotiline
3	F	21	Placebo	Maprotiline	Fluvoxamine
4	M	38	Maprotiline	Placebo	Fluvoxamine
5	F	22	Fluvoxamine	Maprotiline	Placebo
6	F	25	Placebo	Fluvoxamine	Maprotiline
7	F	23	Fluvoxamine	Placebo	Maprotiline
8	M	26	Maprotiline	Fluvoxamine	Placebo

l). 5-Hydroxyindole is not a compound normally found in the circulation and does not co-elute with any other substances tested. The reproducibility (C.V.) of the extraction of 5-hydroxyindole at the concentration used was 5.6% and recovery was between 89 and 94% (mean 92%). The mobile phase consisted of a phosphate buffer (13.6 g of potassium dihydrogenphosphate in 1 l of deionised water, 0.1 *M*, adjusted to pH 5.5 with 5 *M* sodium hydroxide) and methanol (85:15, v/v) to which 0.2 ml/l sodium heptanesulphonate (SHS) was added. All chemicals were purchased from Sigma (Poole, UK). A 5-HT standard (100 μg/l) was prepared in hydrochloric acid (0.1 *M*).

Method

Serotonin was extracted from plasma using 1-ml C_{18} Bond Elut (5-μm ODS Hypersil) columns (Jones Chromatography). Plasma (350 μl) was added to internal standard (5-hydroxyindole, 50 μl) and applied to a column equilibrated with 1 ml of extraction buffer. The column was washed with 2 ml of buffer and then the serotonin eluted with methanol (400 μl). Columns were re-generated with 2 ml of methanol. The eluate was diluted 1:3 with deionised water, and aliquots (20 μl) were injected into the HPLC system with a flow-rate of 1.0 ml/min. Eluates were stored on ice prior to injection.

Detection was by electrochemical detection at an oxidation potential of +0.6 V *vs.* the Ag/AgCl reference electrode. This potential was chosen after investigation of the response of the detector to serotonin at various potentials and construction of a voltammogram. Full-scale deflection was 100 nA, and the peak areas were integrated using the CHROMJET.

Analysis

A four-way repeated measures MANOVA was carried out, the sources of variance being subjects, occasions, days and drugs. This test produces an *F*-value. Results were considered significantly different if $p < 0.05$. Trend analysis was performed on time effects.

RESULTS

The chromatographic conditions achieved an acceptable separation of serotonin from potential interfering subjects within 10 min as shown in Fig. 1. The average retention times for serotonin and the internal standard (5-hydroxyindole) were 3.6 and 6.2 min, respectively. Between-batch precision of a plasma pool stored at −20°C gave a C.V. of 4.1% (mean 121 μg/l, $n = 11$). The method was linear to a serotonin concentration of 700 μg/l. Recovery of added serotonin was 93% at a concentration of 220 μg/l (100 μg/l added to the plasma pool gave a mean result of 214 μg/l). The normal range, previously established from 50 normal subjects, was 70–160 μg/l.

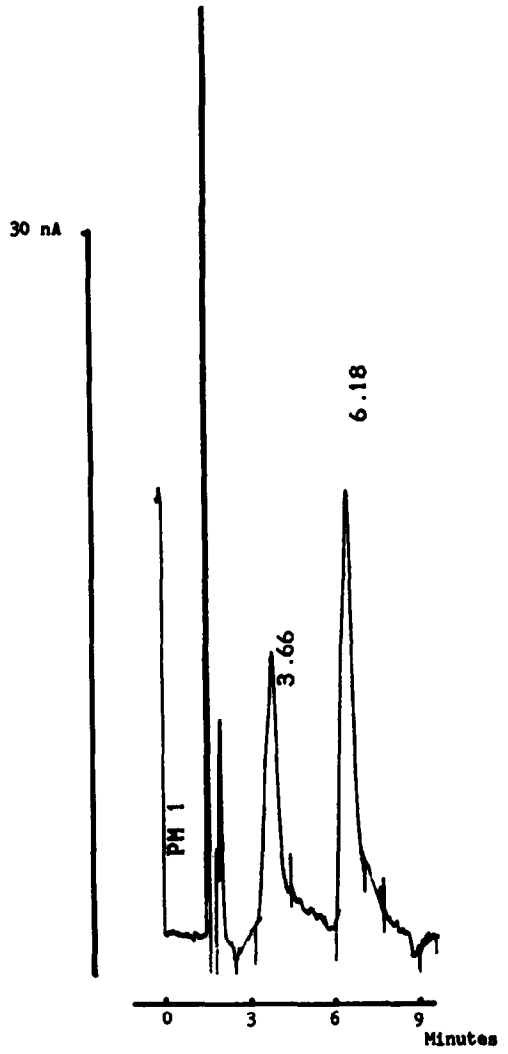

Fig. 1. Chromatographic separation of serotonin. Peaks are serotonin (3.66 min) and internal standard, 5-hydroxyindole (6.18 min).

Subject results

Means and standard deviations for plasma serotonin (μg/l) for the eight subjects on days 1, 4 and 8 of each treatment (placebo, maprotiline and fluvoxamine) are presented in Table II. Although the pre-drug serotonin concentrations (day 1) seem higher for fluvoxamine, they were not significantly different from placebo or maprotiline pre-drug levels. Analysis of pre-drug levels excluded any carryover effects. The results show that fluvoxamine significantly reduced plasma serotonin concentrations over seven days as compared to maprotiline or placebo ($F = 15.54$, $p < 0.0005$). The reduction in serotonin was linear over the time period as shown in Fig. 2. Neither placebo or maprotiline treatment had any effect on plasma serotonin concentrations as shown in Fig. 2.

DISCUSSION

The chromatographic method described for the measurement of plasma serotonin is simple, rapid and specific. Acceptable recoveries of added serotonin were achieved and the reproducibility of the method was good. Linearity was sufficient to cover the range of expected concentrations. The use of solid-phase extraction gives the benefit of removal of potential interfering substances without the disadvantages of liquid–liquid extraction. The combination of extraction and electrochemical detection confers specificity to the method and no apparent interferences were noted. The similarity of the reference range found to those

TABLE II

SEROTONIN PLASMA CONCENTRATIONS IN NORMAL SUBJECTS ON DAYS 1, 4 AND 8 OF SEVEN-DAY TREATMENTS WITH PLACEBO, MAPROTILINE AND FLUVOXAMINE

Concentrations on day 1 and day 8 were compared and the statistical significance is shown (N.S. = not significant, $p > 0.05$).

Treatment	Concentration (mean ± S.D., $n = 8$) (μg/l)			
	Day 1	Day 4	Day 8	
Placebo	121.2 ± 69.8	116.5 ± 56.8	133.1 ± 74.7	N.S.
Maprotiline	125.3 ± 67.6	130.6 ± 70.4	125.3 ± 60.5	N.S.
Fluvoxamine	190.4 ± 105.6	140.4 ± 71.5	71.2 ± 71.5	$p < 0.0005$

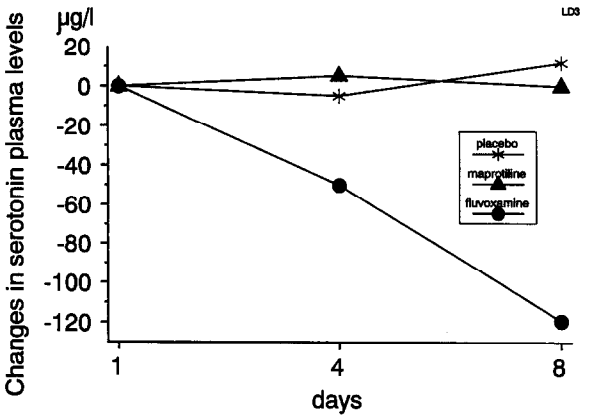

Fig. 2. Effect of fluvoxamine, maprotiline and placebo on plasma serotonin concentrations over an eight-day period. Results are plotted as the change (µg/l) from pre-drug levels.

typically quoted for whole blood confirms the sample preparation stage produces "platelet-rich" plasma.

As expected neither maprotiline nor placebo had any measurable effect on plasma serotonin concentrations. The slightly higher plasma serotonin concentrations in subjects pre-fluvoxamine treatment was not statistically significant and probably represents normal biological variation. Analysis of the data and the variation in order of administration of the three treatment regimens ruled out any carryover effects.

Fluvoxamine significantly decreased plasma serotonin concentrations during the seven-day period of administration to normal volunteers, suggesting that central 5-HT function was augmented by fluvoxamine. This finding is consistent with those of Kremer *et al.* [5] and Hanna *et al.* [6] who reported decreased whole blood serotonin following fluvoxamine administration. Our study suggests that plasma serotonin is equally useful in monitoring the *in vivo* effects of fluvoxamine on serotonin metabolism and has the benefit of being simpler to use. For the assessment of compliance measuring the action of the drug on the target system is likely to be more effective than measuring blood drug concentrations particularly as the effect of fluvoxamine on serotonin was progressive and consumption of a single dose prior to blood sampling whilst likely to produce measurable drug concentrations is unlikely to be sufficient to produce the low plasma serotonin values found during chronic drug use. Kremer *et al.* [5] showed that after two weeks on fluvoxamine the whole blood serotonin concentrations were near the limit of detection of their method and that normal serotonin concentrations were not achieved until three to four weeks after discontinuing fluvoxamine. It is anticipated that if we had continued the study the same would have been true for plasma concentrations.

Reduction of circulating serotonin concentrations have now been reported for a number of the 5-HT re-uptake inhibitors including clomipramine [17], fluoxetine [7,18] and citalopram [19]. Clomipramine reduced platelet 5-HT to 5% of the original value within two weeks and there was a correlation between clinical response and the magnitude of the decrease in 5-HT [17]. Fluoxetine reduced platelet 5-HT to 30% of the original level over a seven-day period [18] and was shown to reduce whole blood 5-HT to 30% of the original concentration over seven days and to 7% after three weeks [7]. These drugs are used to treat patients with depressive disorders and also patients with anxiety disorders [20], both conditions with a high prevalence in the population. From these studies and extrapolating from our results with fluvoxamine to other 5-HT re-uptake inhibitors, measurement of plasma or whole blood 5-HT would seem the most appropriate means of monitoring compliance for all these drugs and potentially the new generation of 5-HT-specific drugs such as paroxetine and sertraline.

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