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Plasma serotonin monitoring by blood microdialysis coupled to high-performance liquid chromatography with electrochemical detection in humans

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Abstract

Plasma serotonin (5-HT) active pool was monitored in male volunteers by intravenous microdialysis coupled to HPLC–EC with 98.6% efficient probes. 5-HT was monitored from 60 min before to 360 min after an oral dose of fluoxetine, a 5-HT uptake inhibitor, or vehicle. The basal values were within nanomolar range (0.55 to 4.6 ng/ml). After administration of fluoxetine, there was a significant increment of 5-HT with respect to controls. These results showed that intravenous microdialysis is an alternative efficient technique to monitor endogenous unbound 5-HT changes in plasma without extracting blood or sample pretreatment procedures before the chemical analysis.

Keywords: Plasma serotonin; Fluoxetine

1. Introduction

Plasma serotonin (5-HT) monitoring is of great importance in clinical and pharmacological research [1,2]. High plasma 5-HT levels are found in carcinoid syndrome patients [3]. The expanding use of serotonergic drugs such as fluoxetine alone or in combination with other psychotropic drugs has increased the risk of serotonergic hyperstimulation [4]. The existence of a plasma 5-HT pool different from the platelet 5-HT pool in rats and human blood has been confirmed [5–7]. Serotonin plasma level (which is less than 1% of whole blood 5-HT) is in the low nanomolar range although with a variability of less than one to several nanograms/ml [5–10]. However, there are marked discrepancies in the 5-HT reported plasma values [8,11–13]. Artifactual release of 5-HT from platelets during blood removal might cause high 5-HT values. Venous microdialysis is an alternative technique to measure plasma 5-HT [14–19]. It could allow direct access to the free 5-HT pool, reducing the probabilities of platelet rupture and improving the accuracy of plasma 5-HT estimation. Moreover, microdialysates are protein free ultrafiltrates that can go directly under chemical analysis. Thus, a continuous chemical monitoring without repeated venous puncture, blood removal or sample pretreatment is possible for every sample minimizing fluid balance alteration [16,19]. In addition, it is an

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easy and safe technique to be used even in outpatients [17–19].

To measure 5-HT in microdialysates very sensitive analytical techniques are required. The coupling of microdialysis almost from the beginning with high-performance liquid chromatography (HPLC) has been very successful. Electrochemical detection (EC) has been preferred to other detection methods [20]. In addition the conventional plasma 5-HT measurements have been done with HPCL–EC [1,7,8].

In the present work, the plasma 5-HT pool was measured by venous microdialysis coupled to HPLC–EC in healthy male subjects before and after a challenge of a single dose of fluoxetine, an antidepressive drug. Fluoxetine is a selective 5-HT uptake inhibitor [21] that enhances serotonergic neurotransmission and the availability of 5-HT in blood [22]. To our knowledge there are no previous reports about acute effects of fluoxetine on plasma 5-HT in humans.

2. Experimental

2.1. Intravenous microdialysis probes

The probes were manually constructed, the details are described elsewhere [19]. In brief, the active area of the removable concentric probes was made of 20-mm long cellulose hollow fiber 220-μm outside diameter and 13 000 molecular mass cut-off (Spectrum Medical Industries, Los Angeles, CA, USA), and a 140-μm outside diameter, 75-μm inside diameter fused-silica capillary (Polymicrotechnologies, Phoenix, AZ, USA). The probes and connections of polyethylene (PE) tubing (Clay Adams, Parsippany, NJ, USA) were individually packed and sterilized with ethylene oxide (Androlene®) for 12 h and ventilated for 24 h before using.

2.2. Subjects

Twenty-four non-medicated healthy male volunteers 16 to 36 years old (mean: 24.5 years), were given a description of the experiments and a written consent was obtained according to the principles of the Declaration of Helsinki and to the ethical standards of our institution.

2.3. In vivo calibration of probes

We used the No Net Flux (NNF) method [19,23] to calibrate the probes for 5-HT in five male healthy volunteers. The perfusion solutions ranged from 0 to 40 ng/ml of 5-HT in sterile 0.9% NaCl solution at a flow-rate of 1 μl/min. Collection of 30-min samples began 90 min after the probe insertion. After changing to a new 5-HT concentration solution, the dialysate of a 10-min period was discarded before starting the collection of the new sample. The 5-HT concentrations in the perfusate solution and in the dialysate were analyzed. The difference between the 5-HT concentration in dialysate and perfusate solution was calculated. A simple linear regression between these differences and the perfusate solution concentration was calculated to determine the NNF point (x axis intercept). This is the estimated unbound concentration surrounding the dialysis membrane. The slope of the regression line gave the recovery value.

2.4. Effect of fluoxetine on plasma 5-HT

Nineteen subjects fasted overnight prior to the session that started at 7 am. The probe connected to the syringe pump (World Precision Instruments, Saratosa, FL, USA) was perfused with sterile 0.9% NaCl solution at 1 μl/min. Under sterile conditions the microdialysis probe was inserted into a venous catheter placed in the cubital vein. After 90 min of equilibration, the collection of a 30-min sample began. Two pre (basal values) and twelve post treatment dialysates were taken. At zero time, the subjects received orally 40 mg of fluoxetine (experimental group $n=10$) or just the vehicle (control group $n=9$).

2.5. HPLC–EC analysis of dialysates

The dialysates were manually injected into an HPLC system equipped with a model 7125 Rheodyne injecting valve with a 20-μl loop and an electrochemical detector (Waters model 464, Mil-

ford, MA, USA). The oxidation voltage was set at +585 mV between a glassy carbon working electrode and a Ag/AgCl reference electrode. The mobile phase was 116.8 m*M* sodium hydroxide, 144.7 m*M* monochloroacetic acid with 100 μ*M* EDTA, 0.69 m*M* octanesulfonic acid and 4.5% (v/v) acetonitrile, adjusted to pH 2.96–3.00. The filtered and degassed mobile phase was delivered by a dual piston pump (Waters, model 515) at a flow-rate of 1.0 ml/min. The separation was performed with a 100 mm long, 3.2 mm I.D., 3-μm particles RP-18 Velosep column (Applied Biosystems, Brownlee Columns, Perkin Elmer, Norwalk, CT, USA). Identification (by retention times) and measurement of the compounds (by peak height) in the samples was achieved by comparison to 20, 50 and 100 pg per 20 μl 5-HT–5-HIAA standard solutions. All chemicals were analytical grade or HPLC grade when possible. They were dissolved in deionized 18.2 MΩ water (Alpha Q Millipore, Bedford, MA, USA). 5-HT and 5-HIAA were prepared as 1 mg/ml stock solution in 0.1 *M* HCl with 100 μ*M* EDTA [20].

Linearity for 5-HT measurement was determined by injecting 10, 20, 40, 50, 60, 80 and 100 pg per 20 μl into the HPLC–EC instrument. Relative standard deviation (R.S.D.) was measured by injecting 10 times a standard solution containing 50 pg/20 μl of 5-HT. The detection limit was established as the mass of 5-HT that generated a signal three times as large as the noise.

2.6. Drugs and reagents

Fluoxetine HCl (Prozac®) from Eli Lilly (Indianapolis, IN, USA). The reagents were purchased from Sigma (St. Louis, MO, USA).

2.7. Statistical analysis

The average basal levels of 5-HT and its metabolite expressed in ng/ml were determined from the two baseline samples before treatment. Statistical analysis of the comparison of experimental and control data was done using one-way analysis of variance (ANOVA) for repeated measures, followed by simple effects comparisons. Significance was set at $p < 0.05$ level.

3. Results

3.1. Analytical procedures

The relationship between the amount of 5-HT injected into the system and the response measured from the height of the corresponding peak was linear from 10 to 80 pg/μl. The simple linear regression equation for this 5-HT calibration line was: $y = 1.1464x$ $r = 0.99$. The R.S.D. was 5.9%, and the minimum detectable amount of 5-HT was 0.89 pg/20 μl.

3.2. In vivo recovery or probe efficiency

In Fig. 1, representative chromatograms are shown obtained from a typical blood dialysate during an in vivo recovery experiment in one individual. 5-HIAA and 5-HT were eluted in that order with retention times of approximately 4 and 7 min respectively. The increment in the peaks is also clear when 5-HT–5-HIAA are added to the perfusion fluid. For the in vivo recovery, the perfusate 5-HT concentration in ng/ml was plotted against the net increase in 5-HT concentration in the dialysate in ng/ml (dialysate 5-HT–perfusate 5-HT) (Fig. 2). The perfusate 5-HT concentration, that did not cause any net increase or decrease in the dialysate 5-HT concentration (the perfusate concentration that was in equilibrium with the surrounding venous 5-HT) was calculated by regression analysis. The simple linear regression equation was: $y = -0.986x + 5.586$, $r = 0.946$. The x intercept value for the No Net Flux point was 5.6 ng/ml. The slope of the regression line gave 98.6 % for in vivo recovery.

3.3. Effect of single oral dose of fluoxetine on plasma 5-HT

The 5-HT means of the two baseline samples in the experimental and control groups ranged from 0.55–4.60 ng/ml, and the means for its metabolite 5-HIAA ranged from 0.70 to 3.50 ng/ml. In Fig. 3 there are representative chromatograms from one individual from the control group and other subject from the experimental group. They have similar basal levels, but after fluoxetine there was a rise in

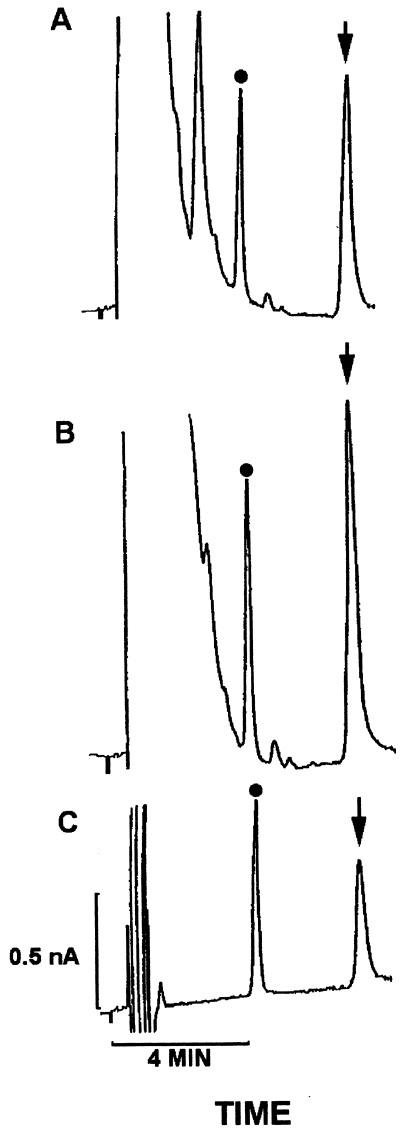

Fig. 1. Chromatographic separation of 5-HT and 5-HIAA in blood microdialysates of the in vivo recovery experiment in one volunteer. The black dot indicates the 5-HIAA peak and the arrow, the 5-HT peak. (A) Without 5-HT–5-HIAA in the perfusion fluid. (B) With 2.0 ng/ml 5-HT–5-HIAA added to the perfusion fluid. (C) 2.5 ng/ml 5-HT–5-HIAA standard solution.

5-HT that was not present after placebo. In Fig. 4 the changes of 5-HT after administration of 40 mg fluoxetine per os are shown. There was a significant increment in 5-HT values compared with the control group $F(1,12)=3.94$, $p<0.001$, from the 210 min to

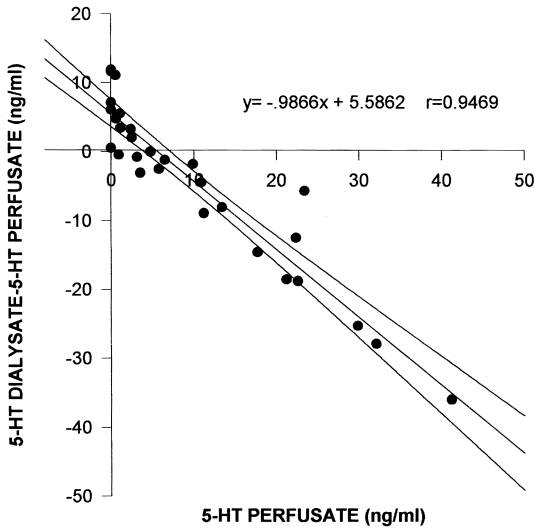

Fig. 2. In vivo recovery of blood microdialysis probes. Plot of the net increase in 5-HT concentration in the dialysate against the perfusate 5-HT concentration. The slope of the regression line gave 98.6%. for in vivo recovery. The zero net flux point was 5.6 ng/ml for these individuals ($n=5$).

330 min, reaching a maximum at 270 min (210 min, $F=4.018$, $p<0.05$; 240 min, $F=5.091$, $p<0.05$; 270 min, $F=11.656$, $p<0.01$; 300 min, $F=8.453$, $p<0.05$; 330 min $F=5.507$, $p<0.05$) and then, the values returned to basal levels. 5-HIAA did not change during the treatment with fluoxetine (Fig. 5). The subjects did not have any complaint during or after the dialysis and felt comfortable with the procedure.

4. Discussion

The present experiments suggest that is possible to access free 5-HT in plasma by intravenous microdialysis coupled to HPLC–EC. The chromatographic conditions allowed a good separation of 5-HT and its metabolite in the samples. The 5-HT basal values in blood dialysates from less than one to a few nanograms per ml are in the low nanomolar range reported by others in platelet-free plasma [5–7]. This agrees with the high in vivo efficiency of our probes (approximately 100%). To our knowledge, this is the first report of acute effect of fluoxetine on human plasma 5-HT. The 5-HT rise in

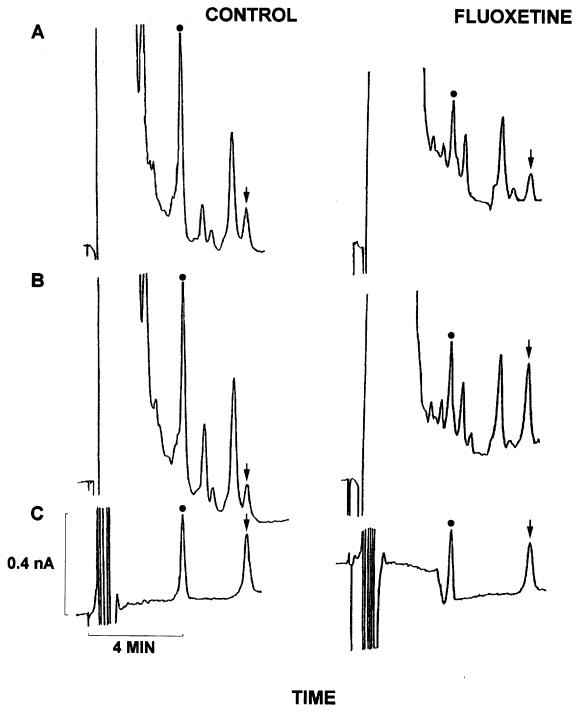

Fig. 3. Chromatographic separation of 5-HT and 5-HIAA in 30-min blood microdialysates from the fluoxetine experiment in two volunteers. The black dot indicates the 5-HIAA peak and the arrow the 5-HT peak. (A) Basal levels of 5-HT and 5-HIAA. (B) 5-HT and 5-HIAA 270 min after placebo (left) or 40 mg per os of fluoxetine (right). (C) 1 ng/ml 5-HT–5-HIAA standard solution.

the dialysates after a single dose of fluoxetine confirms that this 5-HT pool responds to drugs that block the high affinity 5-HT uptake mechanism. This transient effect of fluoxetine agrees with previous reports in rats [16,24]. In the present experiments, the 5-HT metabolite was not affected by fluoxetine, confirming previous observations in rats [16]. 5-HIAA is not considered to be an indicator of 5-HT turnover but of the activity of MAOA [25].

Blood microdialysis might be useful in the monitoring of plasma 5-HT in many different physiological or pathological situations where plasma 5-HT might be involved. For example, patients that receive 5-HT uptake blockers alone or in combination with tricyclic antidepressants or MAO inhibitors might be at risk of developing serotonin syndrome [4]. Interestingly, the percent increase of plasma 5-HT, after fluoxetine administration ranged from 10% to 368 %. This indicates that some people release more 5-HT

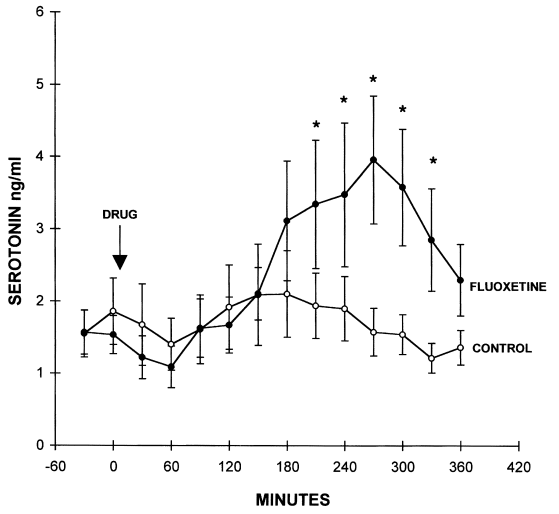

Fig. 4. 5-HT concentration in 30-min blood dialysates. There was a significant increment of 5-HT from 210 to 330 min, after 40 mg per os of fluoxetine ($n=9$) compared with the control group ($n=10$).

than others when receiving fluoxetine. With the techniques reported here, it might be possible to answer the question whether or not the serotonin syndrome occurs preferentially in high plasma 5-HT releasers. In patients with carcinoid syndrome, 5-HT is one of the more relevant active substances produced by the carcinoids tumors, and it has a role in the pathophysiology of many of the symptoms:

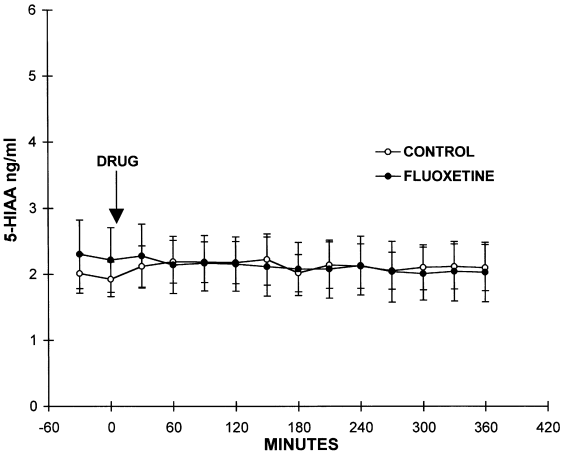

Fig. 5. 5-HIAA concentration in 30-min blood dialysates. There was no increase of plasma 5-HIAA in either the fluoxetine or the control group.

flushing, diarrhoea, dyspnea, and carcinoid heart disease [3]. In the menstrual cycle [10], migraine [26], hypertension [27], cirrhosis [28] toxicity by drugs of abuse such as MDMA (Ecstasy) [29], or diverse psychopharmacologic treatments with serotonin re-uptake blockers, MAO inhibitors, lithium etc. [4] or in psychiatric diseases [1,2]. In all these conditions the plasma extracellular levels of 5-HT might be increased, and monitoring plasma 5-HT should be a useful guide for appropriate treatment.

In the present work, blood microdialysis coupled to HPLC–EC was shown to be an efficient alternative method to monitor platelet-free plasma 5-HT. This technique seems to be a safe and well tolerated method to continuously sample the extracellular compartment with many potential clinical applications in individuals in critical conditions or in healthy volunteers [17–19].

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