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Discussion

Plasma serotonin Reply to E.F. Marshall and M. Leitch

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Serotonin is a well recognized neurotransmitter in the central and peripheral nervous systems. It is implicated to be involved in a number of psychiatric diseases [1,2]. The evidence of the relationships between serotonin and these diseases has been obtained mostly from studies using blood serotonin. Previous data confirmed the existence of a plasma pool of serotonin in two distinctive pools (plasma and platelets) [3–8]. It is agreed that platelets contain 99% of the total serotonin, leaving a small amount in plasma [3–8]. Various methods have been applied for the determination of serotonin in blood, platelets, platelet-rich plasma, platelet-poor or platelet-free plasma [7,8]. Many studies revealed the relationship between psychiatric problems, carcinoid tumors, liver cirrhosis and platelet serotonin [9–11]. We completely agree with the comments by Marshall and Leitch [12]. Indeed, plasma free serotonin may represent the active serotonin in blood and could dictate the sensitivity of the receptors to which it has access. There is a need for a reliably assay of plasma free serotonin although standard procedures for plasma separation, purification steps, and enrichment procedures are lacking [3–11].

In our previous study, we successfully assayed human plasma serotonin by ultrafiltration and microbore high-performance liquid chromatography with electrochemical detection (LC–ED) [13]. The plasma samples were separated with a somewhat questionable separation procedure at 700 *g* for 10 min. Marshall and Leitch pointed out that our plasma samples were, in fact, platelet-rich plasma samples. After the freezing at −70°C and thawing at 4°C procedures the platelets might be ruptured and release their serotonin. Indeed, our serotonin data were much higher (43.86 ng/ml) than those reported previously in platelet-poor plasma (0.6–11.6 ng/ml) [6]. There are four possible reasons for such high values of plasma serotonin. First, the procedures used to separate plasma from blood caused the platelets to break up. Second, the platelets released serotonin or broke up during sample storage (over a 3-months period). Third, centrifugation, freezing or thawing procedures caused the platelets to release their serotonin (or the platelets to break up) into the plasma. Fourth, patients were treated with various drugs, such as 5-HT re-uptake inhibitors, which may elevate plasma serotonin levels. Therefore, in response to the comments of Marshall and Leitch we are investigating serotonin concentrations in platelet-rich and platelet-poor plasma samples during storage at −70°C by microbore LC–ED. Use of microbore LC columns reduced the time required and improved the sensitivity of the analysis substantially.

Venous blood of 33 fasted our-patients was

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Table 1
Plasma free serotonin concentrations in thirty three fasted out-patients

	Free serotonin (mean ± S.E.M.) (ng/ml)			
	1 st day	2nd day	7th day	14th day
5-HT in PRP	1.05 ± 0.33	1.06 ± 0.60	1.07 ± 0.52	1.02 ± 0.54
5-HT in PPP	1.08 ± 0.34	1.24 ± 0.35	1.21 ± 0.32	1.18 ± 0.31

obtained between 8:00 and 9:00 in the morning to avoid possible diurnal fluctuations. The blood samples were collected into pre-chilled polypropylene tubes with heparin as anticoagulant and centrifuged immediately to separate platelet-rich (700 *g* for 10 min at 4°C) and platelet-poor (3500 *g* for 20 min at 4°C) plasma samples [14]. The plasma samples were fractionated and kept at −70°C until assay. After thawing at 4°C in the dark, 100-μl plasma samples were transferred into Millipore Ultrafree-MC units with a 10 000 NMWC cut-off and centrifuged at 15 000 *g* and 4°C for 15 min. A 50-μl aliquot of the ultrafiltrate and 10 μl of Ringer's solution containing ascorbic acid and an internal standard were mixed. Aliquots of (5 μl) the mixture were injected automatically by a CMA-200 refrigerated autosampler onto the HPLC system [13].

Preliminary data (Table 1) indicated that there were no difference in serotonin concentrations between platelet-rich and platelet-poor plasma samples within two weeks. Unlike our previous results in schizophrenia patients (43.86 ng/ml), the out-patient plasma free serotonin data (mean value of 1.05 ng/ml) is very close to those reported previously [6]. Long-term experiments on this subject are currently being performed. We are unable to rule out storage problems (3 months) in our previous data [13]. In addition, there may be some variations between schizophrenia and other patients with complicated drug interactions. Until the separation and the storage problems in the serotonin assays are solved, it turns safe to follow the recommendations by Anderson *et al.* [8] or Marshall and Leitch [12] in the preparation of cell-free plasma and the storage of these samples at −70–−80°C.

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