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Correlation of serotonin levels in CSF, platelets, plasma, and urine

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ABSTRACT

Background: Neurotransmitter levels are best measured in the cerebrospinal fluid (CSF), but that requires an invasive procedure.
Methods: Samples were collected from humans and rats. Eighteen women age 38–51 years with fibromyalgia provided samples of CSF, plasma, platelets, and urine. Samples of CSF, plasma, platelets, and urine were also collected from Sprague–Dawley rats, adult male, 6 months old. One group of rats was treated with p-chlorophenylalanine to decrease their levels of serotonin, and another group of rats was treated with amphetamine to increase their levels of serotonin.
Methodological improvements include: 1) the use of siliconized glassware, plasticware, and tubing to prevent adsorption of serotonin, 2) the extraction of serotonin from the CSF, plasma, and platelets, 3) repeated washing of the platelets with an improved buffer, and 4) early morning sample collection. HPLC/MS was used to measure serotonin after extraction.
Results: For serotonin, the new method of measuring platelet levels resulted in a very high correlation with levels of serotonin in CSF in rats ($r = 0.97$) and humans ($r = 0.97$). There were lower correlations of levels of serotonin in CSF with levels in plasma ($r = 0.77$ for rats and $r = 0.57$ in humans) and urine ($r = 0.67$ in rats and $r = 0.62$ in humans).
General significance: This method of measuring serotonin levels in platelets results in a very strong correlation with levels in CSF, so in most cases platelet measurements will be preferable since it is much less invasive to collect. Levels of serotonin in plasma and urine are significantly but less strongly correlated with levels in CSF.

1. Introduction

Neurotransmitters are essential for normal neurological function, and the level of neurotransmitters can greatly affect mood, learning, memory, and cognition. Brain levels of neurotransmitters can be assessed in cerebrospinal fluid (CSF), but that is a highly invasive procedure. It would be highly desirable to find alternative methods for estimating neurotransmitter levels in the brain.

Several studies have suggested that platelets are good models of neuronal serotonergic cells [1–5]. Both platelets and neuronal cells uptake serotonin by the serotonin transporter (SERT), which transports serotonin across the cell membrane [6]. Serotonin is then stored in dense granules (in platelets) or in synaptic vesicles (in neurons), where the serotonin is bound to saturable binding sites [6]. One study [7] found the dissociating binding coefficient, K_d, for serotonin similar in platelets and neuronal membranes of two strains of rats. In humans, both platelets and serotonergic neurons contain monoamine oxidase B (MAO-B),which is not active against serotonin, and do not contain significant amounts of MAO-A, which does metabolize serotonin [8]; thus, serotonin stored in those cells is relatively stable. So, platelets and serotonergic neurons use the same transporter to carry serotonin into their cell, and similar ways to store it. Both types of cells are major storage sites for serotonin.

Platelets in both humans and in rats were found to contain approximately 25,000 times higher levels of 5-HT than plasma while other catecholamines (epinephrine (EPI), norepinephrine (NE), and dopamine (DA)) were approximately 800 times greater in platelets than in plasma [1]. Several factors can temporarily raise serotonin levels in plasma, and make them less reliable for measurements of serotonin. The consumption of serotonin-rich foods (such as walnuts, hickory nuts, plantains, pineapple, and banana) can substantially increase the level of urinary 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, and may affect plasma levels, but platelet levels are not significantly affected by fluctuations in dietary intake because they contain far higher levels of serotonin [9]. One study found that whole blood serotonin (which is primarily serotonin in the platelets) did not change significantly at 15 min, 30 min, or 60 min after a meal [10]. This is analogous to the measurement of sugar levels in plasma vs. glycohemoglobin, where plasma levels fluctuate much more rapidly than in glycohemoglobin. One study [11] found a high intra-individual correlation of serotonin levels in humans measured 3 months apart, suggesting that platelet levels are a reasonable marker of long-term status.

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Several groups have investigated the possible correlation of serotonin levels in blood vs. CSF/brain [12–15]. Table 1 summarizes those studies. Some studies found very high correlations, whereas others found moderate or no correlation. The reason for the large discrepancy appears to be due to large differences in methodology. One primary problem with measuring serotonin is that after extracting it from biological fluids (CSF, plasma, platelets, urine) it will bind strongly to glassware and plasticware [16]; as will be discussed later, this problem can be largely overcome by the use of silicon coatings. A major problem with using plasma is that it contains extremely low levels compared to platelets, so that any minor contamination by platelets can have a dramatic effect on the results. A challenge with using whole blood is that the platelet concentration can vary; this can be overcome by normalizing by platelet count [17].

The methodology behind 5-HT testing could explain variations in correlation values between cerebrospinal fluid and blood 5-HT. Interference with 5-HT measurements has been reported in radioimmunoassay of CSF with cross-reactions with 5-methoxytryptamine [18], tryptamine [18], and tryptophan [19]. Tryptophan could interfere with 5-HT detection when using chromatography but if the ionic strength of the running buffer was increased to 0.2 M this could be resolved [19]. One study [17] suggests the importance of correcting for platelets when measuring serotonin in blood. They found whole blood serotonin measurements correlated more closely with platelet serotonin when corrected for platelet count. Increased plasma 5-HT concentrations have also been reported if the final centrifugation step is omitted [20]. One study [21] suggests that diet affects measurements of 5-HT. They found lower 5-HT in plasma poor samples for individuals on a tryptophan-poor diet (excluding bananas, chocolate, pineapples, plums, nuts, and mollusks) 24-hours prior to sample collection.

The purpose of this paper is to investigate an improved method for extracting, processing and measuring serotonin in platelets, and to investigate the correlations of levels of serotonin in CSF with levels in platelets, plasma, and urine.

2. Methodology

2.1. Animal study

This portion of the study was approved by the New York University Animal Care and Use Committee. Sprague–Dawley rats, adult male, 6 months old were used for this study. The rats were housed together in groups of eight, and their blood, urine, and CSF were pooled together to generate a single combined sample for testing. Each group of 8 rats was housed in a metabolic cage at 25 °C and fed Lieber and DeCarli liquid diet [22]. The animals were provided with a 12 hour light/12 hour dark cycle, and had access to unlimited water. In order to create a wider range of serotonin levels, one group of rats was given p-chlorophenylalanine to lower their serotonin levels, and another group of rats was given amphetamine to increase their serotonin levels, and a third group was not treated. The medication was given one time by injection into the tail vein.

The rationale for these medications is that laboratory rats have very similar diets, environment, and genes, so therefore they have little variation in their serotonin levels — these medications create a broader range of serotonin values, which is more analogous to the serotonin values in the general human population which has natural variations in diet, environment, and genes.

After the medication was injected, there was a 24 hour waiting period, and then collection of a 24-hour pooled urine sample was begun. Urine was collected every 6 h and the cage was cleaned from fecal pellets at the same time, and the urine from those six-hour collections was pooled over 24 h.

Blood and CSF samples were collected in the morning (8:30–10:30 am) when the 24 hour urine collection was competed (48 h after injection). CSF fluid was collected by a method described previously [23]. After CSF collection, while the animals were anesthetized, blood was collected with siliconized/heparinized syringe from the heart. Blood and CSF levels were withdrawn within approximately 1 h of each other. CSF, blood, and urine were processed as discussed below. Each urine sample was measured in triplicate.

2.2. Human study

2.2.1. Patients

Eighteen female patients age 38–51 years with primary fibromyalgia syndrome were scheduled to provide CSF, urine, and blood for diagnostic purposes for treating their condition, and they gave informed consent to allow their results to be analyzed for this research. Samples were analyzed anonymously in a coded manner, and therefore IRB approval was not required. These patients were all taking a selective serotonin re-uptake inhibitors (primarily prozac (55%) or paxil (35%)), but their psychotropic and analgesic medications were withheld for 24 h before sample collection, but some amount of their medications would still be present in their system. The use of these medications would tend to raise serotonin levels in the CSF.

2.2.2. Sample collection

Blood samples, first morning urine, and cerebrospinal fluid were collected in the morning (8–10 am). CSF was collected from each subject by lumbar puncture between the lumbar vertebrae L3/L4 and L4/L5, and 3 ml was provided for this study when available. None of the CSF samples was contaminated with blood. No patient experienced any adverse event except temporary headache. Blood was collected into a vacutainer tube with EDTA by venipuncture from median cubital vein on the anterior forearm.

Table 1
Summary of 5-HT correlates.

Correlation	Subjects	Method	r=	p=	Reference
5-HT in plasma and CSF	Unmedicated minor surgery outpatients; n=35	HPLC	0.41	<0.02	12
5-HT in whole blood and CSF	Unmedicated minor surgery outpatient; n=35	HPLC	NS	NS	12
5-HT in platelets and brain homogenate	ICR mice	LC-F	0.04	NS	13
5-HT in whole blood and CSF	Japanese primates; n=7	HPLC	0.85 3 am 0.81 9 am 0.64 3 pm 0.54 9 pm	<0.01 <0.01 <0.05 <0.05	14
5-HT in plasma and CSF	Naïve Kunming (KM) mice; n=48	SPE and Nafion/CGSPE by CV. Injection of reserpine	0.80 baseline 0.74–0.93, depending on dosage of reserpine		15

2.3. Sample processing

One general challenge is that serotonin binds to most glassware and plastics [16]. Therefore, all glassware, plasticware and tubing were siliconized to prevent adsorption — this improved reproducibility, sensitivity, and total recovery.

2.3.1. CSF

CSF was dispersed into a polypropylene tube and was mixed with equal volume of 0.1% trifluoroacetic acid (TFA). Hydroxyindolecarboxylic acid (5 ng in 10 µl) was added as an internal standard, and 50 µl of ascorbic acid (1.5 mM) was added. The mixture was centrifuged at 10,000 *g* for 10 min at 4 °C. The supernatant was subjected to strata C18 solid-phase extraction cartridges at 0.5 ml/min. The cartridge was pre-equilibrated with 2 ml of 60% acetonitrile in 0.1% TFA. The column was washed with 0.1% TFA (3 ml) at a flow rate of 1 ml/min. The wash was repeated three times. The neurotransmitters were eluted with 4 ml of 60% acetonitrile in 0.1% TFA (1 ml/min). The eluted neurotransmitters were evaporated to dryness and then kept at −80 °C in darkness. Just before analysis, the extracted neurotransmitters were dissolved in formic acid (30 mM) and methanol with 2% EGTA (100 µl), mixed, and then injected into a HPLC system [24] using a 25 micro-liter sample loop.

2.3.2. Plasma

EDTA plasma was collected and processed in siliconized tubes following the approach of another paper [25]. Plasma was extracted with acid-washed alumina which was carried out as described previously [24] with some modification. The alumina is added to adsorb the neurotransmitters. Briefly, 1 ml plasma is mixed with alumina (10 mg), votexed with 50 pg alpha-methyl dopamine (internal standard) 500 µl Tris buffer (pH 8.5, 1 M) and 2% EGTA. The mixture was subjected to a shaker for 30 min at room temperature and then centrifuged. Alumina was washed twice in this buffer. The neurotransmitters were eluted with 500 µl formic acid (30 min) and methanol (98:2) with 2% EGTA. The eluted sample (100 µl) was injected into the HPLC for measurement [24].

2.3.3. Platelets

Blood was collected in a siliconized vacutainer tube containing EDTA. The whole blood was centrifuged at 200×*g* for 15 min at room temperature to obtain platelet-rich plasma (PRP, approximately 4 ml). PRP was recovered within 1 h after collection. The PRP was made 5 mM with EGTA and centrifuged at 1200×*g* for 5 min at 22 °C to sediment the remaining RBC. The supernatant was separated and again centrifuged at 3200×*g* for 20 min to isolate the platelets. The platelets were suspended in modified Tyrode's buffer containing acetyl salicylic acid (aspirin) at a concentration of 500 ng/ml to prevent aggregation. The buffer is slightly hypertonic, which prevents leakage of serotonin from the platelets for the 30 min required to process the samples; leakage was checked using radioactive serotonin,

Table 2
Correlations of levels of serotonin in CSF, platelets, plasma, and urine for humans and rats.

	CSF	Platelet	Plasma	Urine
Human results				
CSF	1.00			
Platelet	0.97	1.00		
Plasma	0.57	0.60	1.00	
Urine	0.62	0.68	0.74	1
Rat results				
CSF	1.00			
Platelet	0.97	1.00		
Plasma	0.77	0.75	1.00	
Urine	0.67	0.70	0.66	1

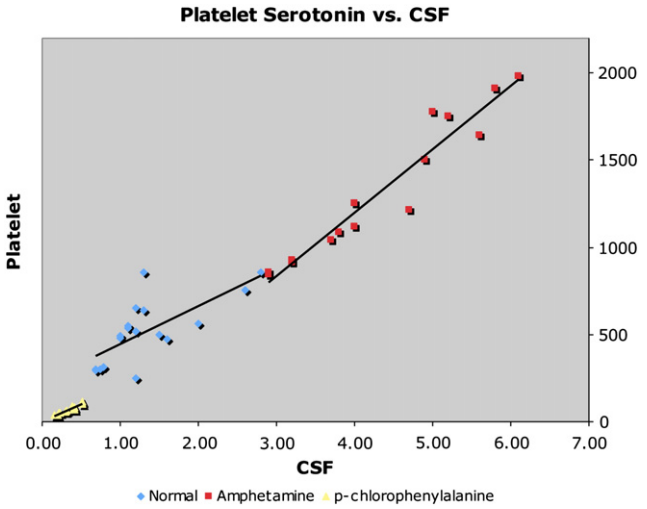

Fig. 1. Platelet levels of serotonin in rats vs. levels in CSF. Units are µg/10^{12} platelets and pg/ml, respectively.

and was found to be non-detectable. The platelets were washed by two successive centrifugation steps (1600×*g*, 10 min) and finally suspended in the modified Tyrode's buffer without EDTA or EGTA, and rested for 15 min at room temperature before use to prevent aggregation. Platelet concentration was adjusted to 10^8 per ml. The entire procedure was performed at 37 °C to prevent aggregation. Isolated platelets were maintained at 37 °C until measured.

2.3.3.1. Extraction of neurotransmitters from platelets. Platelets were incubated at room temperature for 2 h with trypsin (1 mg/ml) followed by soybean trypsin inhibitor (2 mg/ml). Centrifuged as before and washed twice with modified Tyrode's buffer without EDTA or EGTA. Finally the platelets are suspended in sonication buffer (50 mM Tris–HCl, 5 mM EGTA, pH 7.4) containing 50 µl ascorbic acid (1.5 mM) and sonicated by five 20-second bursts (Branson's sonifier, setting 7) followed by centrifugation at 30,000×*g* for 10 min at 4 °C to remove cell debris

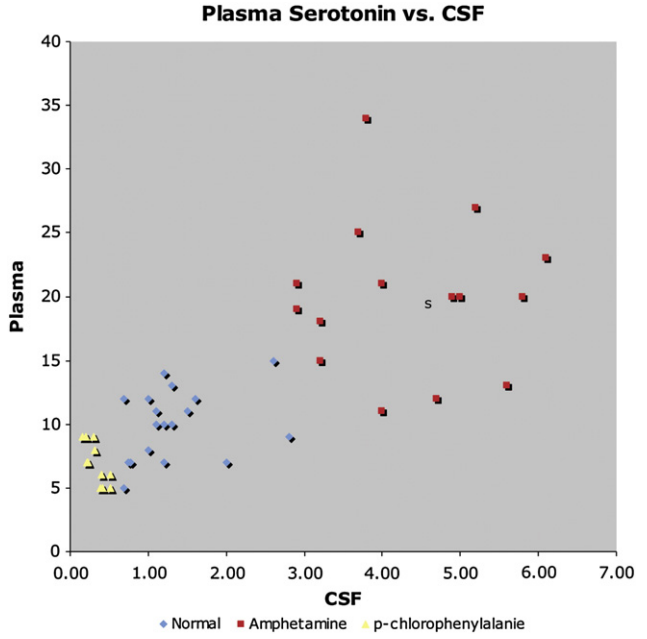

Fig. 2. Plasma levels of Serotonin in rats vs. levels in CSF. Units are ng/ml and pg/ml, respectively.

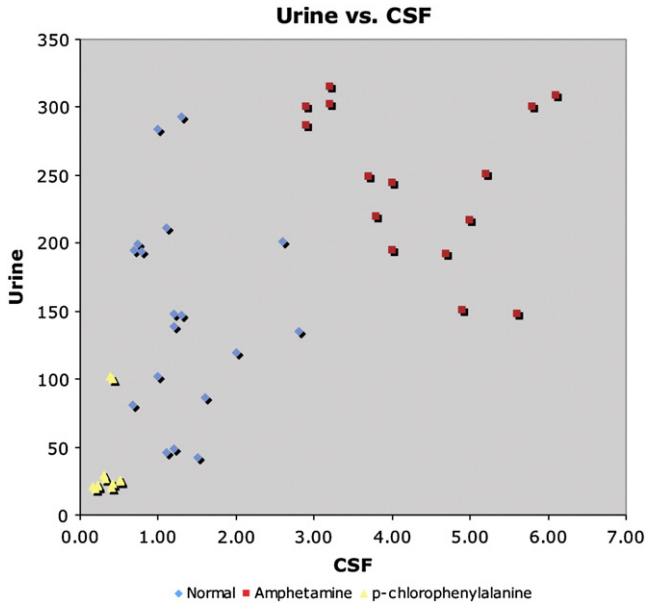

Fig. 3. Urine levels of serotonin in rats vs. levels in CSF. Units are nmole/24 h and pg/ml, respectively.

and unbroken cells. The clear supernatant was isolated after centrifugation, and it was deproteinized by adjusting the pH to 3.0 by the addition of formic acid. The sample was then subjected to HPLC [24].

2.3.4. Urine

All urine samples were processed as described previously [26].

2.4. Analysis of serotonin levels in all samples

HPLC was used to separate serotonin as described previously [24]. Positive ion electrospray tandem mass spectroscopy was used as described previously [26] to measure serotonin levels in all samples (CSF, platelets, plasma, and urine).

2.5. Statistical analysis

Pearson correlation coefficients were obtained to determine the strengths of linear relationships among the variables involved in the analyses.

3. Results

Table 2 shows the correlations of serotonin in CSF, platelets, plasma, and urine for humans and rats. The correlations for the two groups are very similar, with the highest correlations for CSF vs. platelets (r = 0.97 for both humans and rats), and lower correlations for

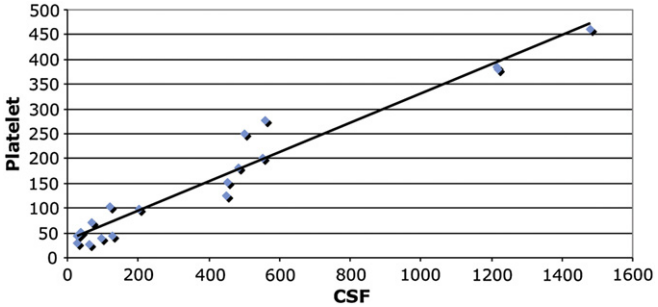

Fig. 4. Platelet levels of serotonin in humans vs. levels in CSF. Units are $\mu g/10^{12}$ platelets and pg/ml, respectively.

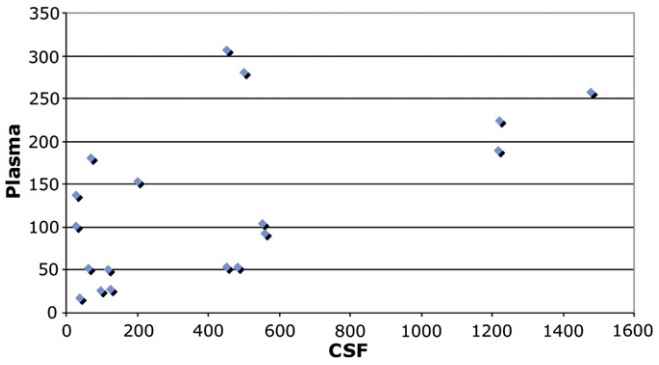

Fig. 5. Plasma levels of serotonin in humans vs. levels in CSF. Units are ng/ml and pg/ml, respectively.

CSF vs. plasma (r = 0.77 for rats and r = 0.57 in humans) and urine (0.67 in rats and r = 0.62 in humans).

Figs. 1, 2, and 3 show plots of serotonin levels in rats for CSF vs. platelets, plasma, and urine, respectively. It is clear that the relationship of levels in CSF vs. platelets is close to linear.

Figs. 4, 5, and 6 show plots of serotonin levels in humans for CSF vs. platelets, plasma, and urine, respectively. Similar to the data for rats, it is clear that the relationship of levels in CSF vs. platelets is close to linear, with much less scatter than for the comparison with levels in plasma or urine.

To further assess the validity of the present method, it is useful to compare the levels of serotonin found in this study with values found in other studies. For CSF, the level of serotonin in the present study is in reasonable agreement with several other studies [12,20,27–30], see Table 3. For platelets, the level of serotonin in the present study is in reasonable agreement with other several other studies [27,31–33], see Table 4. For plasma, the values in the literature are highly dependent on the processing method, because platelets contain far higher levels of serotonin than plasma, so small variations in platelet content can greatly change plasma levels, so it is difficult to compare with other studies that used different processing methods to produce platelet-rich or platelet-poor plasma. For urine, the level of serotonin in the present study is in good agreement with several other studies [34–36], after converting the present units (nmol/24 h) to nmol/mol creatinine and assuming urinary creatinine production is 10 mmol/day [37] (Table 5).

4. Discussion

The strong correlations between serotonin levels in the different samples for both humans and rats suggest that all methods of measuring

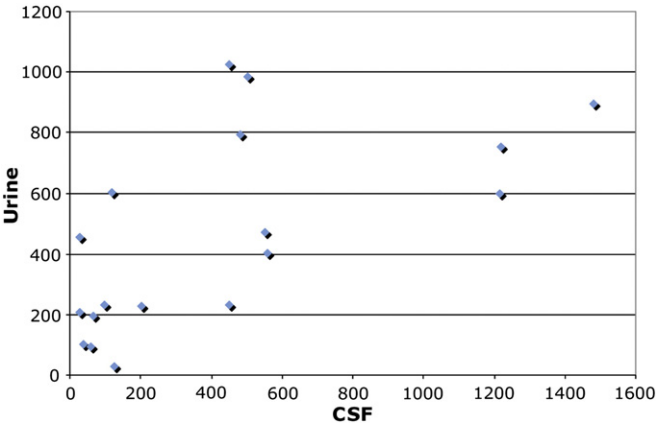

Fig. 6. Urinary levels of serotonin in humans vs. levels in CSF. Units are nmole/24 h and pg/ml, respectively.

Table 3
Comparison of CSF serotonin levels across studies in humans.

Authors	Participants	Mean level of serotonin in CSF (pg/ml), and range or standard deviation, if available
Present study	18 female patients age 38–51 years with primary fibromyalgia syndrome	427 (29–1480)
Artigas et al. 1985 [20]	8 neurological patients (5 Parkinsons, 3 Tourettes)	302 (93–962)
Sarrias et al. 1990 [12]	35 unmedicated minor surgery outpatient	120 (20–2520)
Kumar et al. 1990 [27]	28 healthy adult males	3300 +/− 3400
Kumar et al. 2001 [28]	24 healthy adult men	3530 +/− 3800
Hou C et al. 2006 [29]	80 adults (40 with depression, 40 controls, combined together since no difference between them)	Females: 588 ± 127 Males: 816 ± 321
Kepa et al. 2008 [30]	Healthy controls	3870
Kepa et al. 2008 [30]	Patients with depression and cognitive impairments	1260

Table 4
Comparison of platelet serotonin levels across studies in humans.

Authors	Participants	Mean level of serotonin in platelets (μg/10^{12}), and range or standard deviation
Present study	18 female patients age 38–51 years with primary fibromyalgia syndrome	162 (26–462)
Jernej et al. 2000 [11]	436 healthy males and 64 healthy females aged between 18 and 65 years.	579 +/− 169
Kumar et al. 1990 [27]	28 healthy adult males	748 +/− 448
Ormazabal et al. 2005 [31]	13 young adults	370 (234–539)
Franke et al. 2010 [32]	163 healthy male and female controls	417 (361–519)
Ehrlich et al. 2010 [33]	58 healthy women	472 +/− 162

Table 5
Comparison of urinary serotonin levels across studies in humans.

Authors	Participants	Mean urinary level of serotonin (umol/mol creatinine), and range or standard deviation
Present study	18 female patients age 38–51 years with primary fibromyalgia syndrome	46 +/− 32 *
Kwarts et al. 1984 [34]	8 healthy adult males and 8 healthy adult females	Men: 32.8 (25.5–40.3) Women: 49.3 (40.1–57.1)
Oades et al. 1994 [35]	27 healthy children and young adults	37 +/− 49
De Jong et al. 2010 [36]	120 healthy controls	38.2 (10–78)

* Actual value was measured to be 461 +/− 322 nmole/24 H, which is approximately 46 +/− 32 μmol/mol creatinine, assuming a daily excretion of 10 mmol creatinine [37].

serotonin give somewhat similar results, with by far the highest correlation between levels in CSF and in platelets. This suggests that platelets can be a very reasonable alternative to CSF when measuring serotonin levels, and are probably preferable in most cases since it is much less invasive. It should be noted that the medications given to the animals, and the SSRI medications used by the human participants, would tend to result in a broader range of serotonin levels, which would tend to increase the strength of the correlation.

The present results for a strong correlation between serotonin levels in platelets and CSF are similar to the results for one study [14] for serotonin in whole blood and CSF ($r = 0.54–0.85$), and similar to results of another study [15] for plasma and CSF ($r = 0.74–0.93$). These studies are in contrast to one study [12] which found no correlation of serotonin in whole blood and CSF, and another study [13] which found no correlation between serotonin levels in whole blood and brain homogenate.

It appears that the present method of extracting, processing, and measuring serotonin levels in platelets is superior to that of methods used by some previous researchers (see Table 1), because of 1) the use of siliconized glassware, plasticware, and tubing to prevent adsorption of serotonin, 2) the extraction of serotonin from the CSF, plasma, and platelets (some previous studies measured serotonin directly in those samples without any extraction), and 3) repeated washing of the platelets with an improved buffer to prevent contamination. Also, based on the results of a primate study [14] it appears that early morning provides the strongest correlation between platelet and CSF levels, and that is the time used in the present study for rats and humans, and is probably the best time for testing.

5. Conclusion

Using the present methods of extraction, processing, and measurement, serotonin levels in CSF are strongly correlated with levels in platelets, and less strongly correlated with levels in plasma and urine. The levels of serotonin in CSF, platelets, and urine in this study were in reasonable agreement with those found in several other studies.

Disclosure of financial interests and potential conflicts of interest

TA is the research director at Health Diagnostics and Research Institute, a company which offers laboratory measurements of neurotransmitters in CSF, plasma, urine, and platelets.

JBA does not have any biomedical financial interests or potential conflicts of interest.

LJ does not have any biomedical financial interests or potential conflicts of interest.

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